MUSCLE BIOPSY ANALYSIS OF THE BODY WATER AND ELECTROLYTE CONTENT IN HEALTH AND DISEASE

A thesis submitted to the University of Glasgow for the degree of Doctor of Medicine in the Faculty of Medicine

by

JOHN ALISTAIR GRAHAM

Department of Surgery,
Western Infirmary,
Glasgow, W.1.

June, 1970.

To my wife, Heather.

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ACKNOWLEDGEMENTS

The technique of analysis and partition of the biopsy-specimen water and electrolytes was developed while I was working in the Institute of Physiology, Glasgow University. My supervisor at that time Professor J.F. Lamb, gave me invaluable advice and encouragement, and I wish to accord to him my most grateful thanks. I also wish to thank Professor A.W. Kay who has since continued to give me constant help and encouragement.

I am greatly indebted to Mrs. Irene Thomson of the Institute of Physiology, and also Miss Ann Halliday and Mr. John Brannan of the Department of Surgery, Western Infirmary, who have given me superb technical assistance throughout this work.

Mrs. Elizabeth Nimmo typed the final draft of this thesis quickly and most efficiently, and Miss Ann McCaul made the prints of the figures and some of the tables for inclusion in the text. I wish to thank them both for their invaluable help.

An Aminco-Cotlove chloride titrator was purchased during the course of this work, as a result of a grant made by the committee of the Western Infirmary Endowments Fund, and their financial assistance is gratefully acknowledged.

Finally, I wish to thank the many physicians and surgeons who have made patients under their care freely available to me,

especially Drs. A.L. Linton and A.F. Lever, Professor A.W. Kay and Messrs. A.B. Kerr, K. Fraser and W.G. Scobie.

CHAPTER 1

INTRODUCTION AND HISTORICAL BACKGROUND

INTRODUCTION

The advances in biochemical knowledge in this century have brought about an entirely new understanding of fluid and electrolyte metabolism in health and disease. Although serum-electrolyte concentrations can be measured with accuracy and sophistication using modern automated techniques, it has long been realised that these measurements only give partial information on fluid and electrolyte balance in the extracellular phase of the body. Furthermore, serum-electrolyte values often relate badly to the total body water and electrolyte contents and may even give misleading information. For this reason several methods of estimating total body water and electrolytes have been developed.

Metabolic balance studies were first used to give information on fluid and electrolyte disorders, but the results obtained only give indirect evidence of change, and the studies are laborious, time-consuming and very liable to error except in properly organised metabolic units. The pioneer work of Cuthbertson[1] and Moore and Ball[2] on the metabolic response of the body to trauma is a good example of the important advances arising from the use of this method.

In 1946, Moore published an account of the measurement of body water and solids using the isotope-dilution method[3]. He and subsequent workers have used and modified these techniques

extensively, and the monograph produced by Moore, et. al.[4] describes clearly how such results may be used to assess the fluid and electrolyte balance in individual patients and to dictate therapy. There are, however, many important drawbacks to this method. Incomplete equilibration of the tracer substance, or penetration into body compartments not under study, especially in disease conditions, may seriously confound interpretation of the results. Most of the isotope tracers now used are radio-isotopes, and though the amount of radio-activity given is very small, there is a possible radiation hazard from repeated estimations or from use in infants, children or pregnant women.

Direct analysis of tissue water and electrolytes can also be used to give information on body water and electrolyte contents. Skeletal muscle is most commonly used, as it is the largest single component of the lean body mass, and is of relatively uniform cellular composition. Analysis of a skeletal muscle biopsy-specimen is a simple procedure and does not require complicated or expensive equipment. This technique has been employed in a desultory fashion for many years, but recent work has done much to elucidate the advantages and disadvantages of the method (see historical background).

The purpose of this investigation has been firstly to produce a simplified method of analysis of skeletal muscle biopsy-specimens. Secondly, to study the physiology of water and electrolyte

balance in skeletal muscle from normal adults and children. Thirdly, to enumerate the changes in muscle water and electrolytes in some pathological conditions, and lastly, to re-examine the physiological mechanisms in the light of the changes found in disease states. It is hoped that the results described will allow more knowledgeable and efficient treatment of water and electrolyte imbalance in individual patients.

HISTORICAL BACKGROUND

Some of the early studies on muscle water and electrolytes were carried out using post-mortem materials[5-7] and obviously the results found were open to criticism. Most of the other studies[8-14] had the basic defect that the muscle specimens taken were large, up to as much as 10 G., and naturally it was undesirable to subject patients to this procedure. Also the biochemical analyses were laborious and relatively inaccurate by modern standards. In 1958 Litchfield and Gaddie[15] published an analysis of the water and electrolyte content of small skeletal muscle biopsy-specimens, the total weight of muscle taken being not greater than 300 mg. The reproducibility of the results was high, and the variability was much smaller than that previously found by other workers with larger muscle specimens.

Around this time Flear and his associates started to

analyse biopsy-specimens from large numbers of patients, and from different muscle groups in the body. Their published work did much to establish the technique of the biopsy-specimen analysis[16], and they were able to show that different muscle groups had slightly different water and electrolyte contents[17], confirming differences previously noted in rat muscles[18, 19]. Therefore, in order to compare normal with abnormal, the biopsy-specimens should be taken from the same muscle group in each case.

Rieffel and Stone[20] first described a method of taking muscle biopsy-specimens by needle-biopsy, and their work was developed by Bergström[21]; further modifications to the needle used have been described by Groh, et. al.[22] and by Nichols, et. al.[23]. This method has obvious aesthetic advantages, but does not give as accurate results as open biopsy methods, because the specimen obtained is very small and more liable to contamination with fat and connective tissue. So far, the results obtained by analysis of needle-biopsy specimens have been disappointing in many ways, but further technical advances may eventually make this the method of choice.

Finally it has been shown both in animals[24], adult humans[24] and children[25] that changes in muscle water and electrolytes correlate well with changes in total body water and electrolytes measured by isotope-dilution techniques. It will also be shown in

this thesis that muscle water and electrolyte contents agree with the findings of metabolic balance studies.

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CHAPTER II

BIOPSY TECHNIQUE, ANALYTICAL METHODS AND TERMINOLOGY

BIOPSY TECHNIQUE

Skeletal muscle biopsy-specimens were taken from either vastus lateralis, rectus abdominis or internal oblique muscles. Vastus lateralis was biopsied under both local anaesthesia (2% plain lignocaine) or general anaesthesia (sodium thiopentone, gallamine triethiodide, nitrous oxide and oxygen, and occasionally halothane); the other muscles were biopsied under general anaesthesia alone.

1) Vastus lateralis: the skin and subcutaneous tissues were infiltrated with local anaesthetic; the thick fascia lata prevents diffusion of the solution into the muscle. A 1-3 cm. skin incision was made, the fascia lata was split along the line of its fibres and retracted with pressure forceps, and the epimysium of the vastus lateralis was similarly incised and retracted. The underlying muscle was then gripped in pressure forceps and a biopsy of 100-200 mg. size snipped off with scissors. There is a brief spasm of pain as the muscle is cut, but no residual pain, and it was not necessary to premedicate the patients.

Any excess blood was gently mopped from the specimen, and three small pieces weighing 15-35 mg. were immediately cut from it and placed in weighed 10 ml. centrifuge tubes, and sealed with 'Parafilm' wax sheeting. In this way it was possible to obtain biopsy-specimens free of blood, fat or connective tissue, and also to avoid evaporative water loss.

A number of biopsy-specimens were taken from the vastus lateralis using the same technique, but under general anaesthesia. These were taken within 20 minutes of induction, before the main surgical procedure started.

2) Rectus abdominis or internal oblique: All biopsy-specimens from these muscles were taken within 20 minutes of the induction of general anaesthesia, from the particular muscle exposed by the surgical incision, using the technique described above. The specimen was never larger than 100 mg. in the group of infants and children studied in this way.

ANALYSIS OF THE WATER AND ELECTROLYTE CONTENTS

The fresh specimens in the tubes were re-weighed, after removing the 'Parafilm'. The balance used was accurate to 0.05 mg. The specimens were then dried at 85°C to constant weight (in fact drying for 5 hours ensured complete dehydration). The tubes were allowed to cool to room temperature and then re-weighed.

The electrolytes of the biopsy-specimens were then extracted in 1 ml. 0.05N nitric acid for at least ten hours, preliminary experiments having shown this length of time to be adequate. The extract fluid was then divided as follows:

(i) 0.25 ml. for estimation of the sodium and potassium

contents. This aliquot was diluted 1 in 40, and the electrolytes measured on an 'E.E.L.' flame photometer. The standard solutions contained both sodium and potassium.

(ii) 0.5 ml. for estimation of the chloride content. This was measured using the micropotentiometric method of Ramsay, et. al.[1]. Recently, an amperometric titration method[2] has been used, measurements being made on an Aminco-Cotlove chloridimeter. The two methods give exactly similar results.

PARTITION OF THE WATER CONTENT

The difference between the wet and dry weights of the biopsy-specimen gives the total water content. The simplest method of estimating the extracellular fraction of the total water is to use the endogenous chloride content of the tissue. Most of the chloride is extracellular, but the chloride content of the cells can be calculated easily, since chloride is passively distributed between the cells and the extracellular water according to the cell membrane potential[3, 4]. For a constant membrane potential, taken to be -85 mV[5], it can be calculated from the Nernst equation that the external concentration of chloride bears a linear relation to the intracellular concentration in the proportion of 24 to 1 (see Discussion for further information). Therefore, as the extracellular chloride concentration is known, the intracellular

chloride concentration is easy to find.

The extracellular water of the biopsy-specimen is then calculated as follows:

Since the content of a substance in a given solution equals the volume of that solution times the concentration of the substance in solution, the extracellular and intracellular chloride contents can be described thus:

$$Cl_e = W_e [Cl]_e \qquad \ldots\ldots\ldots (1)$$

$$Cl_i = W_i [Cl]_i \qquad \ldots\ldots\ldots (2)$$

Equation 2 can be rewritten thus:

$$Cl_t - Cl_e = (W_t - W_e) [Cl]_i \qquad \ldots\ldots\ldots (3)$$

Addition of equations 1 and 3 gives:

$$Cl_t = W_e [Cl]_e + (W_t - W_e) [Cl]_i \qquad \ldots\ldots\ldots (4)$$

Solving this equation for W_e gives:

$$W_e = \frac{Cl_t - W_t [Cl]_i}{[Cl]_e - [Cl]_i} \qquad \ldots\ldots\ldots (5)$$

Where,

W_t, W_e, W_i = total, extracellular and intracellular biopsy-specimen water contents.

Cl_t, Cl_e, Cl_i = total, extracellular and intracellular biopsy-specimen chloride contents.

$[Cl]_e$, $[Cl]_i$ = extracellular and intracellular chloride concentrations.

Equation 5 is used to calculate the extracellular water of the biopsy-specimen and all the variables in the equation can be directly measured, except the intracellular chloride concentration which is calculated as above.

CALCULATION OF THE INTRACELLULAR ELECTROLYTES

Knowing the extracellular water content, and the extracellular electrolyte concentrations, the amount of the total biopsy-specimen sodium and potassium contents which is extracellular can be easily calculated. The remainder divided by the intracellular water content gives the intracellular sodium and potassium concentrations.

The extracellular electrolyte concentrations are taken to be the same as the serum-electrolyte values, without applying correction factors for the Donnan equilibrium or the solids content of the serum, since these factors are very small and produce only a negligible effect (in the case of sodium and potassium they cancel each other).

TERMINOLOGY AND REFERENCE BASE

The amount of water and electrolytes in the biopsy-specimens

are multiplied by the appropriate factor to express the results as ml. or meq. per Kg. dry weight of muscle tissue. The dry weight of the muscle was taken as the reference base, as this remains relatively constant throughout changes in water and electrolyte contents of the muscle, and is therefore most suitable for comparison of different samples. In a large number of biopsy-specimens, the mean fat content was only 3% and individual variation around this mean was very small. The estimation of fat in the specimens also introduces a further manipulation and therefore a further source of error into the analysis. For these reasons the fat content of the samples has not been measured, and therefore the reference base dry weight includes a small amount of fat (see Discussion).

The intracellular electrolytes are expressed as meq. per litre intracellular water.

DISCUSSION - ADVANTAGES AND POSSIBLE DISADVANTAGES OF THE METHOD

The main advantage of this method is its ease of application. Skeletal muscle biopsy-specimens may be taken quickly and almost painlessly after very little practice. The analysis of the samples is technically easy, and only requires apparatus readily available in most hospital biochemical laboratories; it

is therefore inexpensive. The results are obtained quickly (16 - 20 hours at present), considerably faster than even the most sophisticated isotope-dilution techniques.

Only endogenous substances are measured by muscle biopsy. This therefore excludes the doubts present with isotope-dilution techniques as to equilibration of the tracer or penetration into compartments not under study. Also, more accurate information can be obtained by muscle biopsy in acute clinical states; no time is required for equilibration of tracer substances, time during which the pattern of the imbalance may be quickly changing.

It is possible using this method to estimate the intracellular water and electrolyte content of the muscle cells, the largest component of the lean body mass. This is especially of interest in the study of the physiopathology of a disease process. Calculated "intracellular" electrolyte concentrations can be obtained using isotope-dilution methods, but the meaning of such figures is difficult to interpret.

The technique of analysis of the biopsy-specimens and the calculation of the results differs in some respects from other studies; these changes have been made so that the method is as simple as possible. Firstly it has been shown that the technique of handling the specimen avoids significant evaporative water loss. It is not necessary therefore to take serial weighings of the

biopsy-specimen, as had been previously advocated[6, 7]. Other workers have shown this also, using different techniques[8, 9]. Secondly, the fat content of the samples has not been estimated, as fat appears to constitute only a small and relatively constant fraction of the solid content of the biopsy-specimens. Flear, et. al.[10] claim to have found up to 21% fat content in muscle samples, but this seems excessively high and is probably due to significant visible fat contamination of the specimen, or to loss of non-fat tissue during the fat extraction.

The main objection against biopsy-specimen analysis of water and electrolytes is whether the results obtained are an accurate reflection of the total body water and electrolytes. Welt, et. al.[11] believe that as muscle tissue represents the largest single tissue mass in the body, its changes are highly significant as a reflection of changes in total body composition. Good correlations have been shown between the results of muscle biopsy analysis and those of isotope-dilution studies[12, 13]. Wherever possible in this study the changes found by muscle biopsy have been checked by metabolic balance studies, and agreement has been good (see details with specific cases in later chapters). It seems likely therefore that the changes from normal found by muscle biopsy-specimen analysis do reflect changes taking place in the total body constituents.

The inherent difficulty of all methods of studying body

water and electrolytes is to find an accurate method of measuring the extracellular water content. In this method the endogenous chloride content of the biopsy-specimen is used as the indicator substance. Numerous workers used the total chloride content of the tissue to estimate the extracellular water, without making allowance for any intracellular chloride (see Chapter 1 for references). However, muscle cells do contain some chloride[14, 15], and it is also believed that the intracellular content varies in pathological conditions[3, 15, 16]. The total chloride content would therefore not be an accurate indicator of the extracellular space under all conditions.

Conway[3] and Hodgkin and Horowicz[4], in a wide variety of experimental conditions, showed that the chloride content of muscle tissue was passively distributed between the cells and the extracellular water, according to the cell membrane potential. This finding has been used in this study to calculate the intracellular fraction of the total chloride content in the light of the extracellular chloride concentration. This contributes a worthwhile increase in the accuracy of the estimation of the extracellular water content, both in health and disease.

However, this difference in the method has in itself posed a further possible inaccuracy, as the calculation of the extracellular water will be accurate only if it can be assumed

TABLE 1

EFFECT OF MEMBRANE POTENTIAL ON

INTRACELLULAR CHLORIDE CONCENTRATION

MEMBRANE POTENTIAL (mV)	CALCULATED INTRACELLULAR CHLORIDE (meq. per l.)	EXTRACELLULAR WATER (ml. per Kg. dry wt.)	DIFFERENCE (%)
-75	6.0	753	-5.9
-85	4.2	800	-
-95	2.9	831	+3.7

Calculated for extracellular chloride concentration of 100 meq. per l; total biopsy-specimen chloride content 90 meq. per Kg. dry wt; total water content 3200 ml. per Kg. dry wt.

that the membrane potential does not vary by more than ± 10 mV from the assumed value of -85 mV; Table I shows that changes of membrane potential of this magnitude introduce only a small error to the calculations. In a letter published after the initial account of this method[17], Flear, et. al.[18] contended that membrane potential measurements from individual muscle fibres in normal subjects may vary by as much as 34 - 48 mV, and rightly pointed out that the variations of this magnitude would introduce serious error to the calculation of the intracellular chloride concentrations. However, there are two important objections to this statement. Firstly, there is bound to be variation around the mean in electrophysiological measurements; obviously a muscle biopsy will sample a large number of individual fibres with variable membrane potentials and therefore different intracellular electrolyte concentrations. The important finding will be the mean membrane potential of the fibres and the mean intracellular electrolyte concentrations. Secondly, the results quoted by Flear, et. al.[18] come from papers by Johns[19] and Creutzfeldt, et. al.[20], who measured the membrane potential of human muscle cells <u>in vivo</u>, and both authors stress the difficulties and limitations of this method. The assumed membrane potential used in this study was taken from the work of Elmqvist, et. al.[5, 21] who produced an elegant and completely non-traumatised preparation of human intercostal muscle

which they were able to perfuse with oxygenated physiological solution during recording. The 95% confidence limits of their results are only 8.8 mV on either side of the mean. Johns[19] in fact believes that the low membrane potentials recorded from one of his subjects were almost certainly due to muscle damage; the mean value for his other subjects is 86 ± 6 mV (± one S.D.). Therefore the use of this membrane potential in the study of normal subjects would appear to be justified.

The cell membrane potentials may however be outwith the range of ± 10 mV in abnormal cases. The only published reports on membrane potential measurements in man are in neurological disorders. Creutzfeldt, et. al.[20] found membrane depolarisation in a patient with episodic adynamia, but neither they nor Shy, et. al.[22] found significant change from normal in familial periodic paralysis, even during paralytic attacks. The membrane potential is normal in myasthenia gravis[5]. On the basis of <u>in vivo</u> electrophysiological measurements, it is unlikely that changes in membrane potential of more than 10 mV from normal will be found in fluid and electrolyte imbalance in clinical practice. For instance, it is well known that depolarisation of cardiac muscle cells by more than 10 mV renders them inexcitable[23, 24], a situation incompatible with life <u>in vivo</u>! Prasad[25] found that the membrane potential of human atrial muscle cells bathed in potassium-free

solution was only hyperpolarised by 8 mV on average. Cardiac and skeletal muscle cells have very similar membrane potential values and respond similarly to changes in their external environment. Rector, et. al.[26] produced gross potassium deficiency states in rats, and found hyperpolarisation of skeletal muscle cell membrane by an average of 8 mV. Although results such as these should be applied in the in vivo situation in man with caution, they tend to enhance the indirect evidence available in some metabolic disorders, that the cell membrane and cell function are remarkably normal. Particular examples of such evidence will be given later in the appropriate chapters of this thesis. Until an experimentally sound method to measure membrane potential in vivo is developed, it is necessary to use whatever evidence is available, and at present there is little to suggest that gross changes in membrane potential are likely in most fluid and electrolyte disorders.

SUMMARY

The technique by which muscle biopsy-specimens were obtained in this study has been described, and the methods of analysis are discussed. The method differs in several respects from previous work, in an attempt to simplify analysis.

The corrected chloride content of the biopsy-specimens is used as an indicator of the extracellular water. The

intracellular electrolytes can then be calculated.

The dry weight of the muscle is used as the reference base as this is least likely to alter in pathological conditions. The fat content of the specimens is a small and relatively constant fraction of the dry weight, and has therefore not been measured.

The advantages and possible disadvantages of this method are discussed. The available evidence suggests that it is a satisfactory method, and that the water and electrolyte contents of the biopsy-specimens reflect the water and electrolyte contents of the body.

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NOTES ON STATISTICAL ANALYSES AND TERMINOLOGY

The statistical methods used in this thesis were all taken from Industrial Experimentation, H.M.S.O., London, 1957.

Student's t test was used to calculate the significance of differences between the means of groups of ill patients and the normal mean values. The value of t in each case includes the correction factor $\sqrt{n_1 \times n_2 \div n_1 + n_2}$ (where n_1, n_2 are the number of samples) to take allowance of differences in the number of samples studied.

The significance, P, of the values of t and of the correlation coefficients, r, were taken from the appropriate tables of Documenta Geigy Scientific Tables, 1962.

The 95% confidence limits of the biopsy-specimen normal mean values are taken to be the normal ranges of these values ($\pm$ 2 standard deviations on either side of the mean). Individual biopsy-specimen results in ill patients are therefore said to be significantly different from normal, or to lie outwith the normal range, when a particular value lies outside the 95% confidence limits of the normal mean value.

On the other hand, if the mean biopsy-specimen value of a group of ill patients is said to be significantly different from normal, this statement is made on the basis of a Student's t test

analysis, the P value of t being at least less than 0.05.

CHAPTER III

MUSCLE WATER AND ELECTROLYTES IN NORMAL ADULT SUBJECTS

INTRODUCTION

Graham, et. al.[1] were first to publish the results of muscle biopsy-specimens from normal subjects using the method described in the previous chapter. Biopsy-specimens were taken from vastus lateralis muscle under local anaesthesia, and from rectus abdominis under general anaesthesia; the total potassium content and the intracellular potassium concentration were significantly higher in the vastus lateralis group.

The possible reasons for this finding were firstly that other muscle groups in the body have been shown to have slightly different water and electrolyte contents[2], and secondly that the ionic composition of the muscle had been altered by general anaesthesia, as previously reported[3, 4].

In addition to the main purpose of formulating normal standards for the study of muscle water and electrolytes in disease, this study has also been carried out to decide which of these alternatives was the explanation of the previous finding.

PATIENTS AND METHODS

Muscle biopsy-specimens were taken, analysed and partitioned as described in Chapter II.

The subjects had all been admitted to hospital for minor

TABLE II

MEAN SERUM-ELECTROLYTE VALUES FOR

THREE GROUPS OF NORMAL SUBJECTS

GROUP	SERUM ELECTROLYTES (meq. per l. or mg. per 100 ml.)				
	SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA
VASTUS L.A.					
MEAN	142	4.7	102	26	34
(n=24) S.D.	5	0.4	4	2	4
VASTUS G.A.					
MEAN	141	4.6	102	26	34
(n=18) S.D.	4	0.3	4	2	8
RECTUS G.A.					
MEAN	140	4.5	102	27	32
(n=18) S.D.	4	0.4	4	2	5

S.D. = standard deviation

L.A. = local anaesthesia

G.A. = general anaesthesia

elective surgical procedures. All had normal serum-electrolyte values and patients with any disorder likely to cause electrolyte imbalance were excluded. Some of the subjects, previously reported as normal[1] have been excluded from this study, as they had conditions now known to have a possible effect on fluid and electrolyte balance.

The biopsy-specimen results have been divided into three main groups:-

(1) 24 specimens taken from the vastus lateralis under local anaesthesia (vastus L.A. group).

(2) 18 specimens taken from the vastus lateralis under general anaesthesia (vastus G.A. group).

(3) 18 specimens taken from the rectus abdominis under general anaesthesia (rectus G.A. group).

RESULTS

Individual serum-electrolyte concentrations were all within the normal range, taken as:- sodium, 137 - 148 meq. per 1; potassium, 4.1 - 5.5 meq. per 1; chloride, 96 - 106 meq. per 1; bicarbonate, 24 - 31 meq. per 1. Table II shows the mean serum-electrolyte values for the three groups; none of the differences were statistically significant.

The mean age and range of the subjects in these groups

TABLE III

MEAN BIOPSY-SPECIMEN RESULTS FOR VASTUS L.A. AND VASTUS G.A. GROUPS,

AND THE STATISTICAL SIGNIFICANCE OF THE DIFFERENCES BETWEEN THE GROUPS

GROUP		BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTE (meq. per litre)		
		TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORID
VASTUS L.A. (n=24)	Mean	3169	733	2436	143	420	86	17	171	4.3
	S.D.	150	125	170	28	31	14	7	8	0.2
VASTUS G.A. (n=18)	Mean	3210	644	2546	127	440	78	13	172	4.3
	S.D.	192	140	178	24	31	13	8	7	0.1
DIFFERENCE										
t VALUE		0.777	1.678	2.037	1.938	2.055	1.932	1.643	0.405	0.000
P VALUE		N.S.	N.S.	< 0.05	N.S.	< 0.05	N.S.	N.S.	N.S.	N.S.

N.S. = not significant

were:- vastus L.A. group, 51 (22 - 86) years; vastus G.A. group, 44 (18 - 60) years; rectus G.A. group, 43 (24 - 69) years. There were 8 females in the first group, 6 in the second and 4 in the third.

Table III shows the mean values for muscle water and electrolytes in the two vastus groups of subjects. The results for each individual subject are the average of triplicate estimations on one biopsy-specimen; so the total variability of the average values shown in Table III is composed of the difference between subjects, and the difference between the triplicate estimations from each subject. Analysis of variation reveals however, that the variability shown as the standard deviation in the Table is largely due to a real difference between individual subjects, and only slightly to variation between the biopsy-specimen estimations in each individual subject.

Table III also gives the statistical significance of the differences between the two vastus lateralis groups; the intracellular water and potassium contents are higher in the vastus G.A. group and just reach statistical significance at the 5% level. This difference would not be expected as a result of anaesthesia, and is almost certainly due to the lower mean age of the vastus G.A. group. Moore, et. al.[5] showed changes in body water and electrolyte contents when their normal subjects were divided into

TABLE IV

BIOPSY-SPECIMEN RESULTS FROM VASTUS LATERALIS DIVIDED INTO AGE-GROUPS

AGE-GROUP		BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
		TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
16 - 30 YEARS (n=6)	MEAN	3126	670	2457	131	430	78	16	174	4.3
	S.D.	113	61	125	31	34	7	8	9	0.4
31 - 60 YEARS (n=29)	MEAN	3221	709	2512	135	431	83	14	171	4.3
	S.D.	175	152	186	27	31	15	7	7	0.3
61 - 90 YEARS (n=7)	MEAN	3097	709	2388	145	416	83	18	173	4.2
	S.D.	144	109	177	30	38	13	9	10	0.3

None of the differences are statistically significant.

TABLE V

AVERAGE VALUES FOR ALL VASTUS LATERALIS BIOPSY-SPECIMENS

VALUE		BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
		TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
	MEAN	3187	703	2483	136	429	82	15	172	4.3
(n=42)	S.D.	168	135	180	27	32	14	7	8	0.2
95% CONFIDENCE LIMITS		2851-3523	433-973	2123-2843	82-190	365-493	54-110	1-29	156-188	3.9-4.7

TABLE VI

MEAN BIOPSY-SPECIMEN RESULTS FOR THE VASTUS LATERALIS

GROUPS COMPARED TO THE RECTUS G.A. GROUP

GROUP		BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTE (meq. per litre)		
		TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
VASTUS TOTAL (n=42)	MEAN	3187	703	2483	136	429	82	15	172	4.3
	S.D.	168	135	180	27	32	14	7	8	0.2
VASTUS L.A. (n=24)	MEAN	3169	733	2436	143	420	86	17	171	4.3
	S.D.	150	125	170	28	31	14	7	8	0.2
VASTUS G.A. (n=18)	MEAN	3210	644	2546	127	440	73	13	172	4.3
	S.D.	192	140	178	24	31	13	8	7	0.1
RECTUS G.A. (n=18)	MEAN	3119	639	2484	137	408	75	19	164	4.3
	S.D.	143	142	160	20	23	14	6	11	0.2

TABLE VII

STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN VASTUS LATERALIS AND RECTUS ABDOMINIS BIOPSY-SPECIMENS, CALCULATED BY STUDENT'S T TEST

GROUP	BIOPSY-SPECIMEN WATER			TOTAL BIOPSY-SPECIMEN ELECTROLYTES			INTRACELLULAR ELECTROLYTES		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
RECTUS v. VASTUS TOTAL	N.S.	N.S.	N.S.	N.S.	$P<0.02$	N.S.	N.S.	$P<0.005$	N.S.
RECTUS v. VASTUS L.A.	N.S.	$P<0.05$	N.S.	N.S.	N.S.	$P<0.02$	N.S.	$P<0.05$	N.S.
RECTUS v. VASTUS G.A.	N.S.	N.S.	N.S.	N.S.	$P<0.005$	N.S.	$P<0.05$	$P<0.02$	N.S.

N.S. = not significant

into three age groups, 16 - 30 years, 31 - 60 years and 61 - 90 years. Table IV shows the average values for water and electrolyte contents for the subjects of the vastus L.A. and vastus G.A. groups combined, when divided into age groups as above. The total water, intracellular water and total potassium content all tend to be lower in the oldest age group, although no differences are statistically significant. The differences between the vastus groups are not therefore likely to be due to an effect of general anaesthesia but to an age difference (when subjects aged over 60 are excluded the mean results are very similar), and the results of the vastus L.A. group and the vastus G.A. group have been combined, and the average values will be used as the normal standards in this study (Table V).

Table VI shows the mean results of the vastus lateralis groups of biopsy-specimens compared to those of the rectus G.A. group, and Table VII shows the statistical significance of the differences found. The potassium content and intracellular potassium concentrations are higher in all the vastus groups (note especially between vastus G.A. and rectus G.A., where the anaesthetic used and the mean age of the groups were similar). The extracellular water and chloride contents are higher in the vastus L.A. group, and the intracellular sodium concentration is lower in the vastus G.A. group. There would appear therefore to be consistent differences between the muscle water and electrolyte content of vastus lateralis

and rectus abdominis, and these differences are not due to the type of anaesthetic used or to the age of the subjects.

There are not enough females in the groups to permit analysis of any possible sex differences in water and electrolyte contents.

The results so far may be summarised thus:

(1) The potassium content and intracellular potassium concentration is significantly lower in rectus abdominis compared to vastus lateralis. As all the biopsy-specimens in abnormal cases were taken from vastus lateralis, the findings in rectus abdominis will not be discussed further, except in relation to the findings in normal infants and children (See Chapter IV).

(2) The differences between the vastus L.A. and vastus G.A. groups are almost certainly due to the lower mean age of the vastus G.A. group. However, as the age differences found are not statistically significant, the total material of the two groups can be combined, and the mean values obtained taken as the normal reference standards. The correlations given below refer only to the results from this combined group of 42 subjects.

Correlations

No correlations were found between any serum-electrolyte concentration and the corresponding biopsy-specimen content or

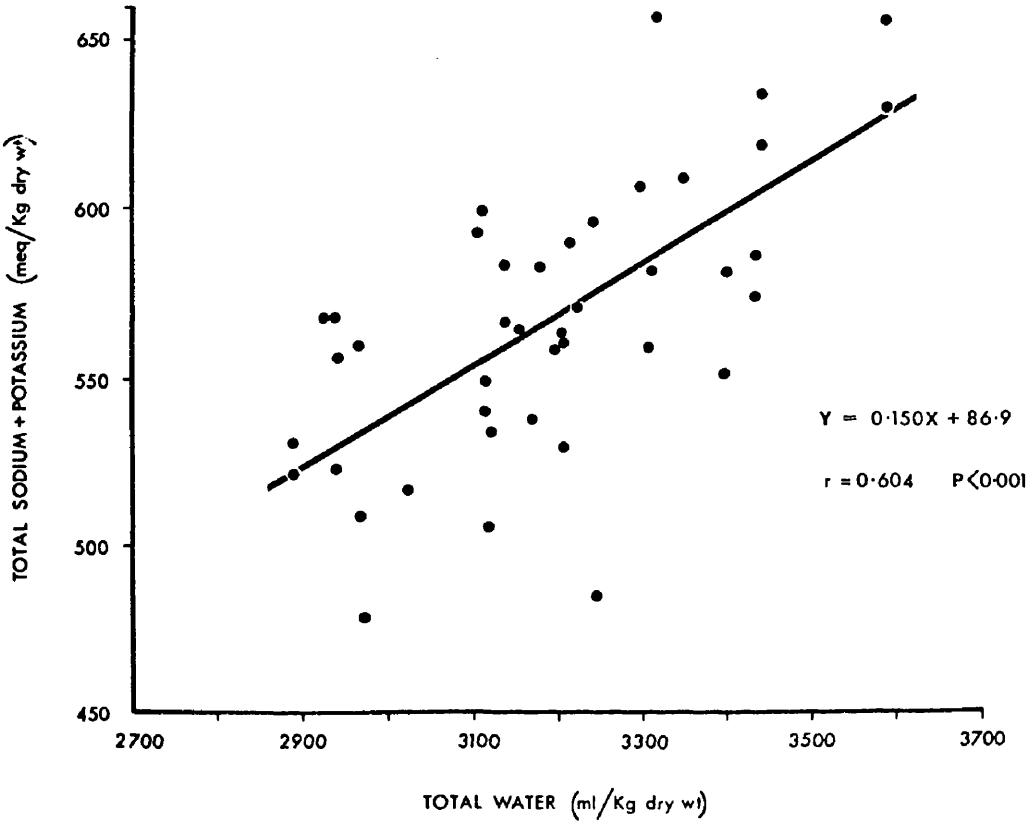

Figure 1: Correlation between biopsy-specimen total water and the sum of the total sodium and potassium contents in 42 normal subjects.

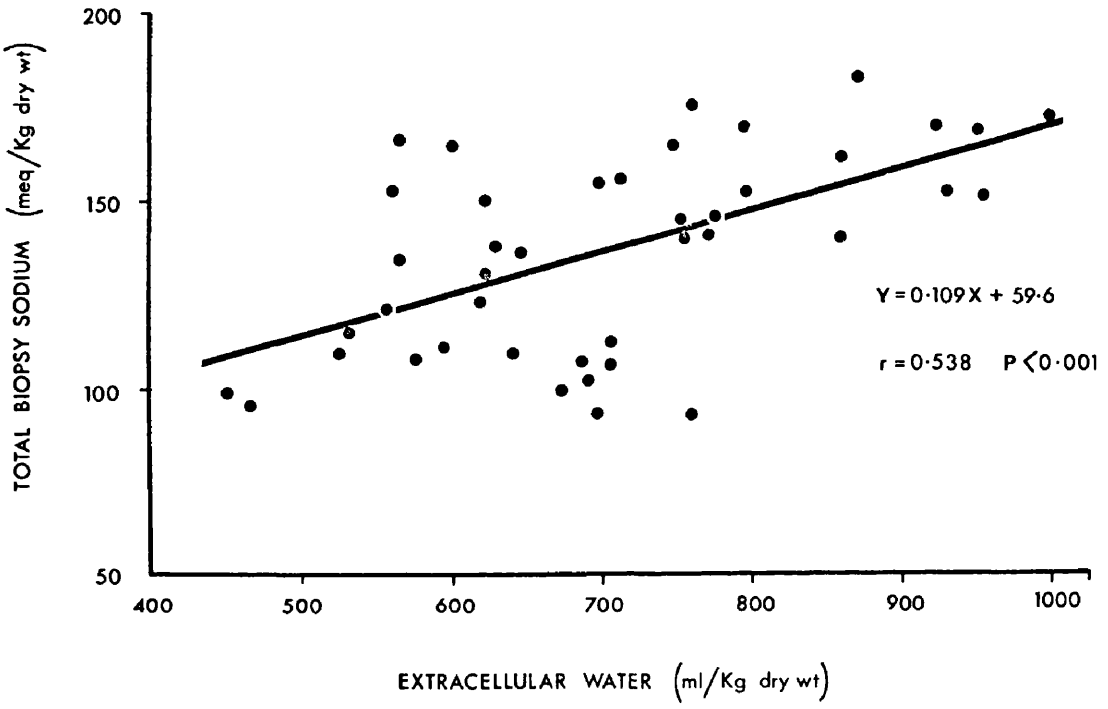

Figure 2: Correlation between biopsy-specimen extracellular water and total sodium content in 42 normal subjects.

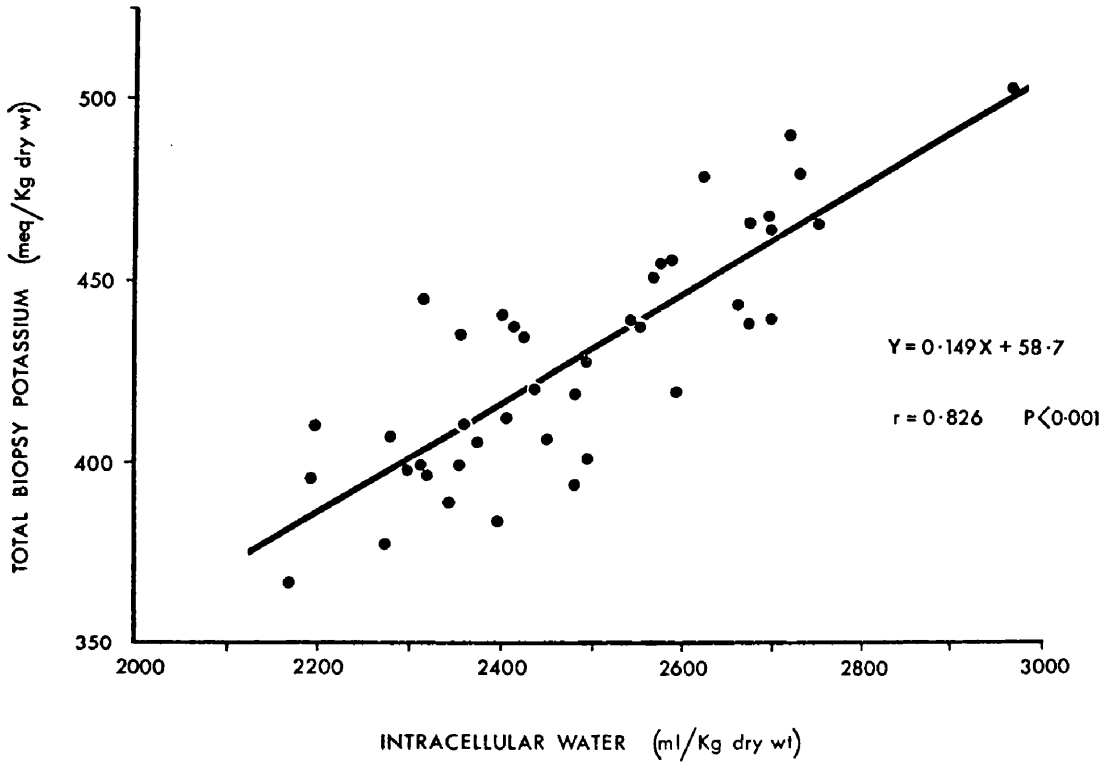

Figure 3: Correlation between biopsy-specimen intracellular water and total potassium content in 42 normal subjects.

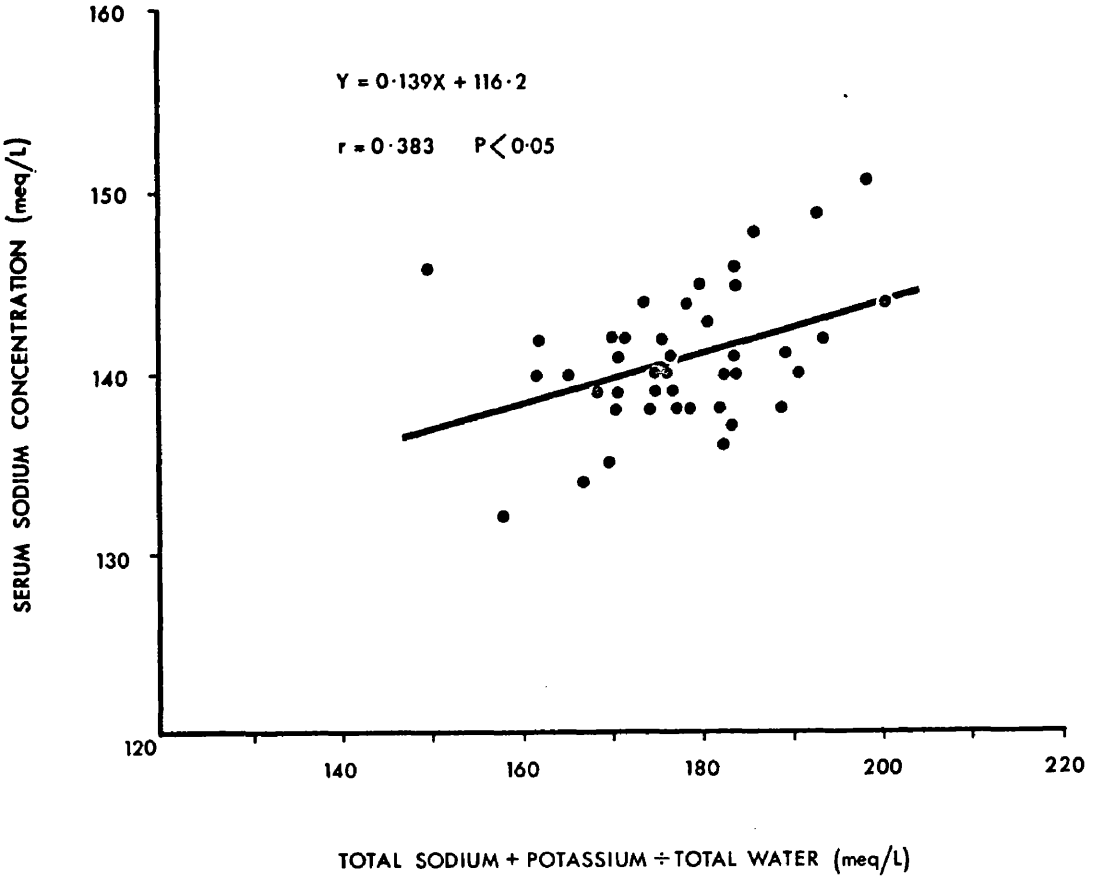

Figure 4: Correlation between total cation concentration and serum sodium concentration in 42 normal subjects.

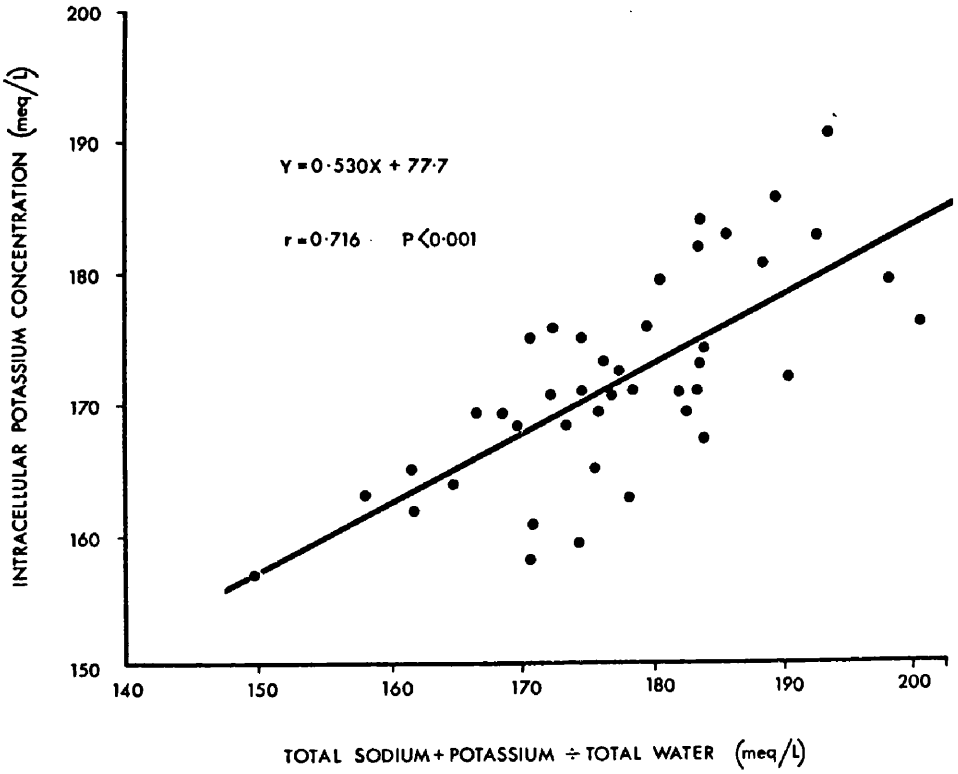

Figure 5: Correlation between total cation concentration and intracellular potassium concentration in 42 normal subjects.

intracellular concentration.

There was a strong correlation between biopsy-specimen total water content and the sum of the sodium plus potassium contents (Figure 1). There were better correlations between extracellular water and sodium content (Figure 2) and between intracellular water and potassium content (Figure 3).

There was a poor, but statistically significant correlation between the serum sodium concentration and the total cation concentration of the biopsy-specimens (i.e. sodium plus potassium contents divided by total water), shown in Figure 4. There was a good correlation between the intracellular potassium concentration and the total cation concentration (Figure 5). These correlations are an indication of the osmotic homogeneity of the body (see discussion).

DISCUSSION

The results of the original groups of biopsy-specimens presented here confirm the findings of Flear, et. al.[2] that different muscle groups have slightly different water and electrolyte contents. The lower potassium content and intracellular potassium concentration found in rectus abdominis compared to vastus lateralis are real differences and are not due to the effects of anaesthesia or the age of the subjects.

TABLE VIII

COMPARISON OF BIOPSY-SPECIMEN RESULTS OF PRESENT SERIES WITH PREVIOUS REPORTS

SERIES	NO. OF PATIENTS	VALUE	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
			TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
MOKOTOFF, et. al.[9]	4	MEAN	3557	570	2987	116	466	70	12	157	-
		S.D.	-	-	-	-	-	-	-	-	-
TALSO, et. al.[10]	16	MEAN	-	-	-	-	-	-	17	153	-
		S.D.	-	-	-	-	-	-	8	8	-
CORT & MATTHEWS[11]	3	MEAN	3500	-	-	132	455	85	5	169	-
		S.D.	-	-	-	-	-	-	-	-	-
WILSON[12]	8	MEAN	3449	-	-	181	408	115	13	166	-
		S.D.	132	-	-	26	32	23	9	20	-
BARNES, et. al.[13]	10	MEAN	3140	-	-	81	469	-	26	150	-
		S.D.	-	-	-	-	-	-	12	11	-
NICHOLS[14]	6	MEAN	-	-	-	185	421	112	13	156	-
		S.D.	-	-	-	40	28	32	4	5	-
LITCHFIELD & GADDIE[15]	23	MEAN	3390	690	2700	139	447	81	10	164	-
		S.D.	110	112	146	19	25	14	6	12	-
BERGSTRÖM[21]	46	MEAN	3340	730	2730	116	447	87	5	167	-
		S.D.	198	203	210	27	20	24	3	12	-
PRESENT SERIES	42	MEAN	3187	703	2483	136	429	82	15	172	4.3
		S.D.	168	135	180	27	32	14	7	8	0.2

Other authors have related their findings to the wet weight of muscle tissue and have therefore not been included. The reference numbers are taken from the bibliography at the end of Chapter I.

Muscle tissue is known to gain water, sodium and chloride and to lose potassium after lengthy surgical procedures under general anaesthesia[3, 4]. The vastus G.A. group had lower sodium and chloride, and higher potassium contents than the vastus L.A. group; these changes are unlikely therefore to be due to an effect of general anaesthesia.

It has been shown both in animals[5, 6] and humans[7, 8] that intracellular water and potassium fall with increasing age. The results of this study tend to confirm these findings, although no statistically significant change could be shown. However, the differences between the two vastus groups seem almost certain to be due to the greater mean age of the subjects in the vastus L.A. group, and the total material of the two groups has been combined. The normal values for biopsy-specimen water and electrolytes so obtained, and the variability, are similar to the results of other workers, (Table VIII).

Edelman, et. al.[9] first reported the correlation between total body water and the sum of the exchangeable sodium and potassium contents of the body. This was to be expected as the sum of the sodium and potassium contents represents about 95% of the osmotically active cation of the body, the principal determinant of the "water holding property of the body"[8]. As sodium is mainly extracellular, and potassium intracellular, it should also be expected that

correlation will be present between extracellular water and sodium content, and intracellular water and potassium content. All these correlations have been shown to hold good for skeletal muscle in this study.

It has also been shown that the serum sodium concentration (the principal extracellular cation) and the intracellular potassium concentration (the principal intracellular cation) both relate to the total cation concentration of the biopsy-specimens. This gives good evidence for an osmotic balance being maintained between extracellular and intracellular phases of the tissue. The correlation of serum sodium to total cation concentrations is not strong, but Edelman, et. al.[9] showed that it was necessary to include material from cases with abnormal serum sodium concentrations, in order to 'stretch' the range and produce a good correlation. This relationship will therefore be studied later, with the inclusion of the results from abnormal cases. The correlation of intracellular potassium to total cation concentration is much stronger, but will likewise be examined under abnormal conditions in a later chapter of this thesis.

All these correlations suggest that, at least under normal conditions, any change in intracellular potassium content is balanced osmotically by movement of water in or out of the cell, rather than by movement of sodium to keep the cell cation content

normal, as had been previously described[10, 11]. Movement of water, rather than replacement with sodium, has the effect of keeping the concentration of potassium in the cell within relatively narrow limits. As the normal membrane potential of the cell depends on the ratio between the concentrations of internal and external potassium, this would appear to be a more suitable homeostatic mechanism than replacing potassium with sodium, which will cause a reduction of the cell membrane potential, and therefore a drop in efficiency of the cell function.

SUMMARY

Muscle water and electrolyte contents have been analysed in 60 normal subjects.

The mean biopsy-specimen potassium content and intracellular potassium concentration are lower in rectus abdominis compared to vastus lateralis.

There is no effect of general anaesthesia on muscle water and electrolytes if biopsy-specimens are taken soon after induction of anaesthesia.

There may be a slight reduction in intracellular water and potassium content with increasing age, but the differences are not statistically significant.

Serum-electrolyte values do not correlate with the appropriate biopsy-specimen content or intracellular concentration.

Strong correlations are found between the total water and the sum of the sodium and potassium contents; between extracellular water and sodium contents; intracellular water and potassium contents; and the intracellular potassium concentration and the total cation concentration. There is a poor correlation between serum sodium concentration and the total cation concentration. These correlations reveal the osmotic homogeneity of the body, and suggest that changes in cell potassium content are balanced by changes in intracellular water content and not by reciprocal changes in intracellular sodium content.

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CHAPTER IV

MUSCLE WATER AND ELECTROLYTES IN NORMAL INFANTS AND CHILDREN

INTRODUCTION

Body water contents of infants and young children have been measured using indicator dilution techniques[1-6]. Exchangeable electrolyte contents have also been measured using these techniques[7-9]. However, as already stated, indicator-dilution studies have limitations, and there may be a possible radiation hazard in the administration of radio-isotopes to children.

Body water and electrolytes have also been measured by muscle biopsy analysis, but early results[10-15] tended to ignore the variation in water and electrolytes with age, as shown by Metkoff, et. al.[16], Dickerson and Widdowson[17] by post-mortem muscle analysis, and by the isotope-dilution studies mentioned above.

This chapter therefore gives the findings of muscle biopsy studies carried out on a series of normal children. The results presented have been reported by Graham and Scobie[18].

PATIENTS AND METHODS

Thirty-three normal children have been studied, ranging in age from 2 weeks to 9 years. They had all been admitted to hospital for elective surgical procedures and none had clinical evidence of fluid and electrolyte balance or any disease process likely to cause this. The details of the procedure were explained

TABLE IX

COMPARISON OF BIOPSY-SPECIMEN RESULTS FROM SUBJECTS MORE THAN 2 YEARS OLD,

WITH VALUES FOR NORMAL ADULTS TAKEN FROM RECTUS ABDOMINIS

GROUP		BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
		TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
CHILDREN AGED 2-9 YEARS (n=9)	MEAN	3325	747	2579	154	424	88	20	165	4.3
	S.D.	336	128	320	19	36	11	7	11	0.2
ADULTS (n=18)	MEAN	3119	639	2484	137	408	75	19	164	4.3
	S.D.	143	142	160	20	23	14	6	11	0.2.

to all the parents and their consent was freely given. It was considered unethical to carry out a vastus lateralis biopsy on these children and therefore a specimen was obtained from a muscle exposed by the surgical incision (rectus abdominis or internal oblique in all cases).

The results obtained have been compared to the results of biopsy-specimens from adult patients (age 24 - 69 years) taken from rectus abdominis muscle under general anaesthesia.

Biopsy-specimens were taken, analysed and partitioned as described in Chapter II.

RESULTS

Attempts to analyse the results of the biopsy-specimens into age-groups showed that over the first two years the values are changing rapidly. From 2 years onwards, however, there appears to be very little change, and the mean values for all such subjects have an acceptable standard deviation. Table IX compares the mean results of this group with the results of the biopsy-specimens taken from the rectus abdominis of normal adults. The total water, sodium and chloride contents are significantly greater in the children at the 5% level; none of the other differences are statistically significant.

TABLE X

INDIVIDUAL BIOPSY-SPECIMEN WATER AND ELECTROLYTES FOR SUBJECTS UP TO 2 YEARS OF AGE

Age (Months)	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
0. 5	5143	1769	3373	310	614	199	20	180	4.3
0.75	4119	1101	3017	233	494	129	26	162	4.4
1.25	3740	1040	2700	209	473	116	21	173	4.3
1.25	3695	1122	2573	226	466	130	27	179	4.4
1.75	3266	1077	2188	170	362	119	10	163	4.3
1.75	3732	1012	2720	205	468	128	22	170	4.3
3. 0	3831	839	2992	155	490	100	13	162	4.3
5. 0	3811	1182	2629	201	448	130	16	169	4.2
5. 0	3689	727	2911	170	441	98	23	151	4.5
7. 5	3314	789	2524	152	391	96	16	153	4.5
7.75	3219	666	2553	188	397	94	36	154	5.1
7.75	4482	956	3526	228	564	121	27	159	4.6
8. 0	3319	1148	2171	218	407	126	24	187	4.3
8. 5	3335	840	2495	186	416	96	27	166	4.3
10. 0	3426	942	2483	148	411	112	8	164	4.5
10. 0	3136	610	2536	134	447	79	19	167	4.6
12. 0	3309	881	2428	176	412	105	23	168	4.5
15. 0	3933	1185	2747	211	428	133	19	154	4.3
16. 0	3369	862	2507	134	418	105	6	165	4.5
18. 0	3265	927	2337	167	459	114	16	194	4.6
20. 0	3505	1055	2449	205	391	122	26	159	4.4
21. 0	3052	716	2336	156	451	86	25	191	4.4
24. 0	3664	1004	2659	172	420	113	15	157	4.2
24. 0	3402	944	2458	186	460	109	19	185	4.3

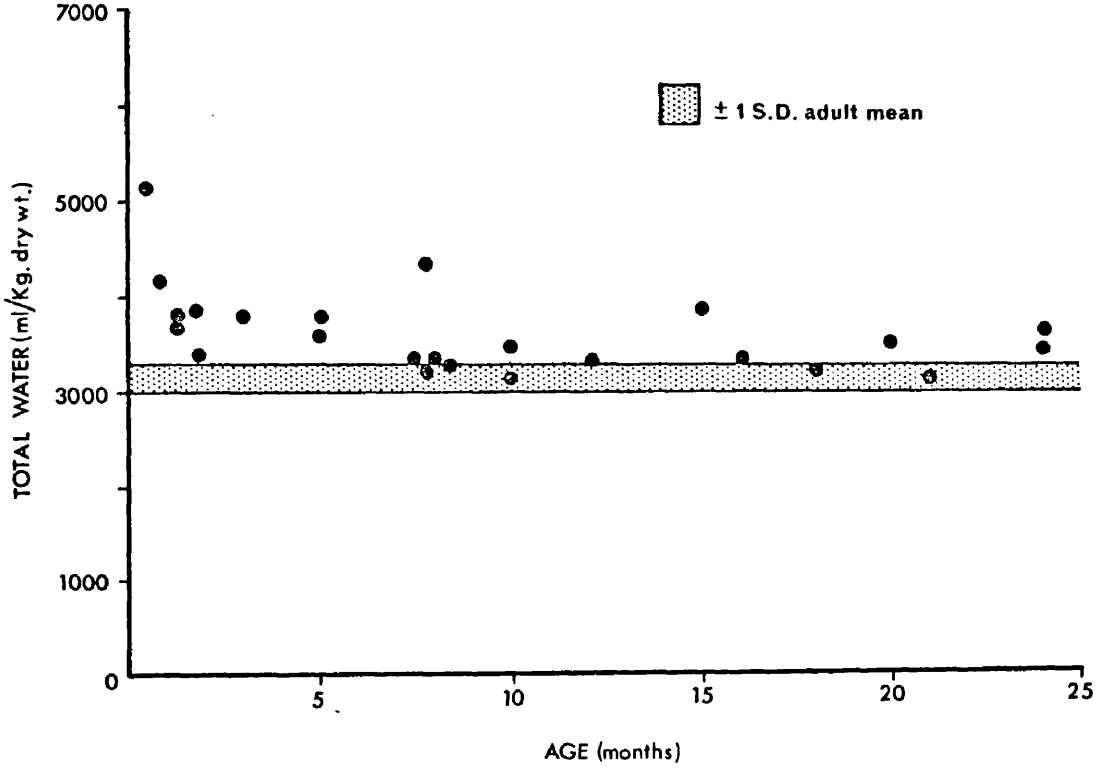

Figure 6: Biopsy-specimen total water as a function of age in 24 children under 2 years of age, compared to ± 1 S.D. normal adult mean.

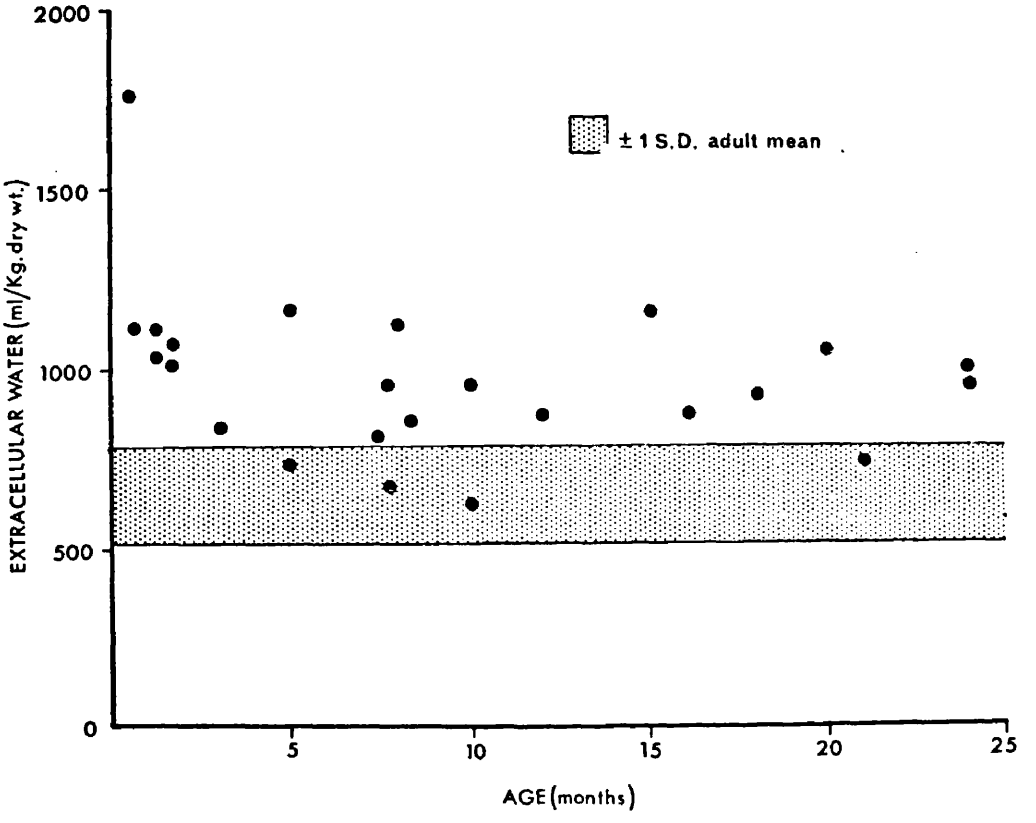

Figure 7: Biopsy-specimen extracellular water as a function of age in 24 children under 2 years of age, compared to $\pm$ 1 S.D. normal adult mean.

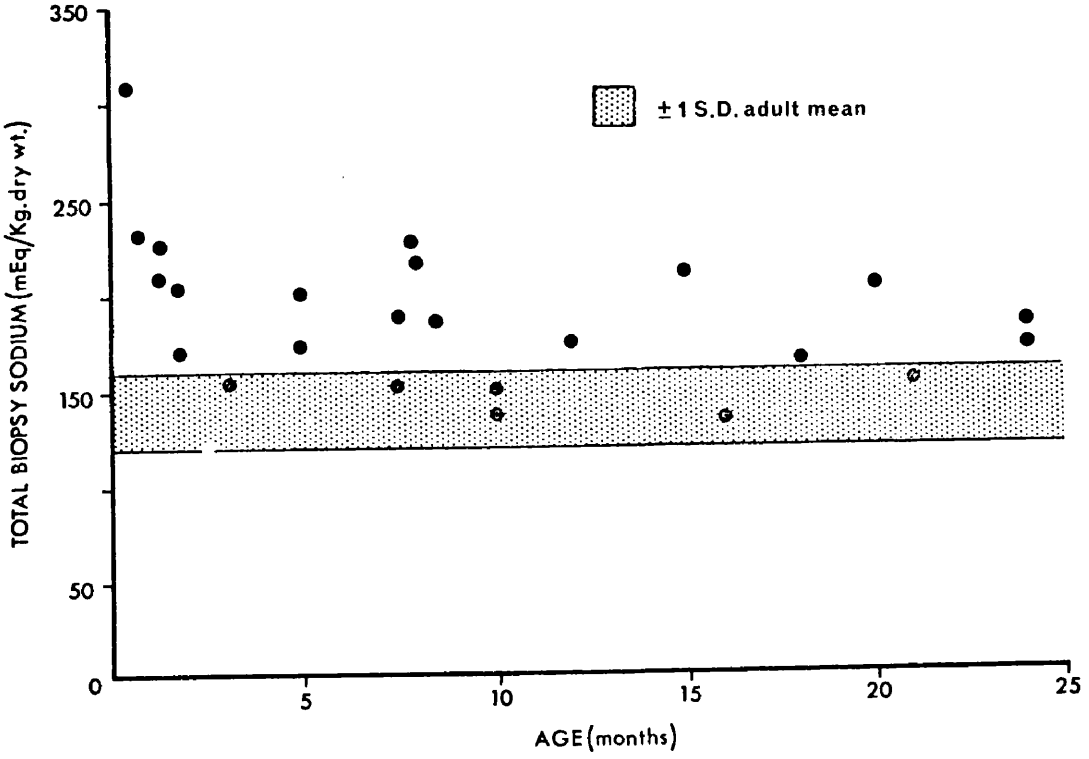

Figure 8: Total biopsy-specimen sodium content as a function of age in 24 children under 2 years of age, compared to $\pm$ 1 S.D. normal adult mean.

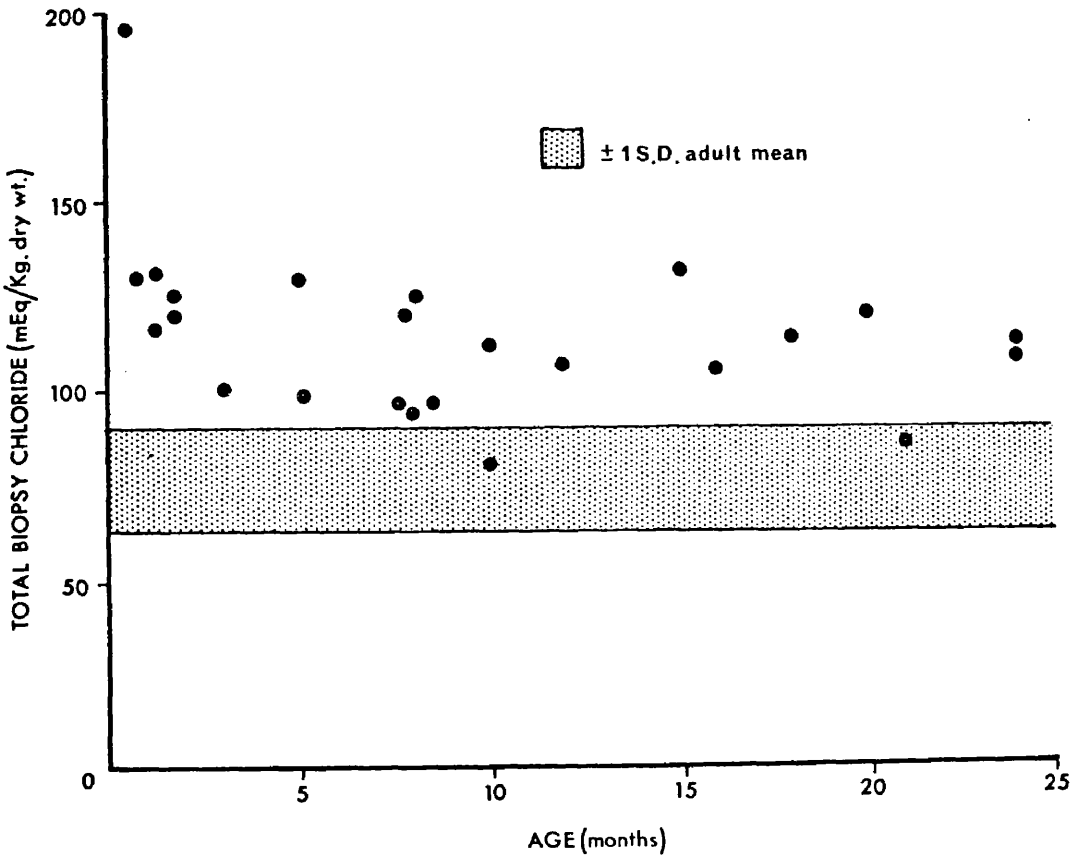

Figure 9: Total biopsy-specimen content as a function of age in 24 children under 2 years of age, compared to ± 1 S.D. normal adult mean.

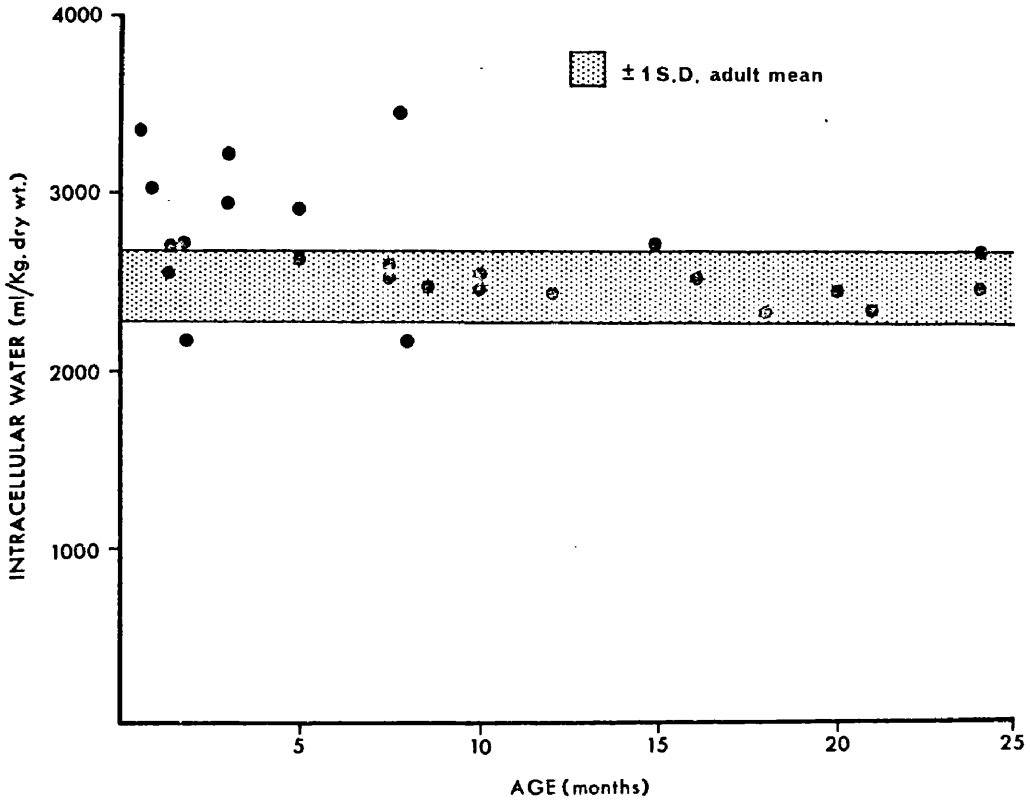

Figure 10: Biopsy-specimen intracellular water as a function of age in 24 children under 2 years of age, compared to ± 1 S.D. normal adult mean.

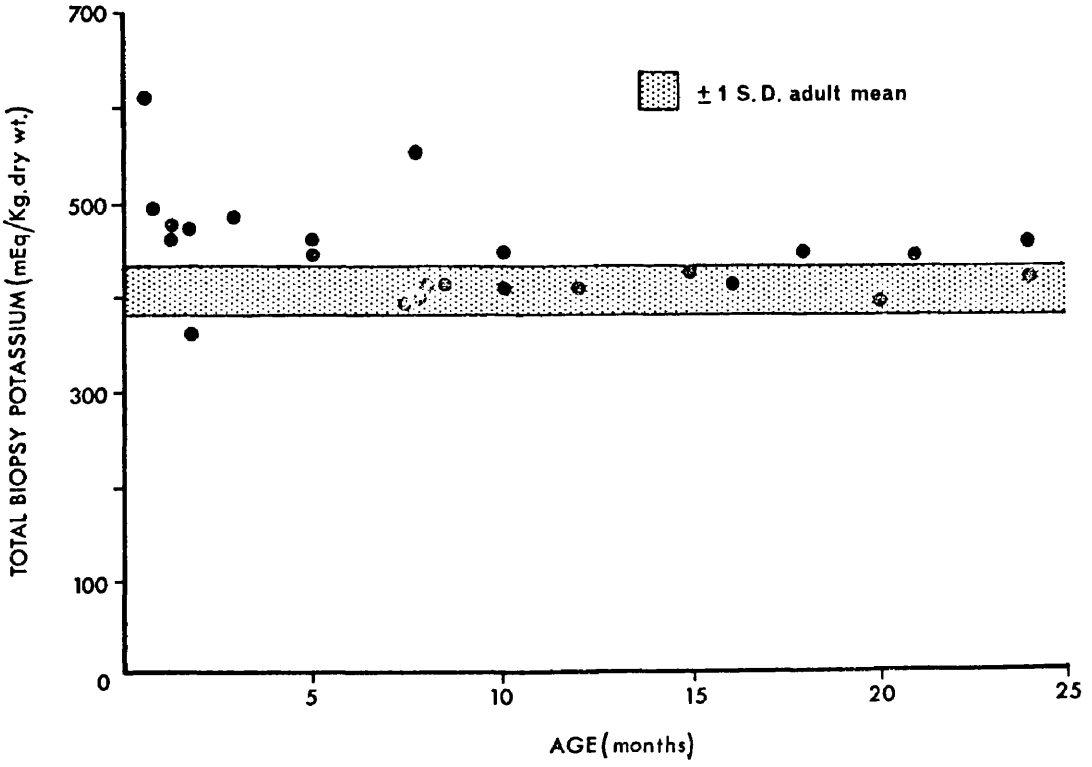

Figure 11: Total biopsy-specimen potassium content as a function of age in 24 children under 2 years of age, compared to $\pm$ 1 S.D. normal adult mean.

Individual biopsy-specimen results for all subjects below 2 years of age are shown in Table X. Serum-electrolyte values are not given, but were normal except for slight elevation of the urea concentration in 7 cases. There was no clinical evidence of abnormality in these subjects and they have therefore been included as normal; possible causes for this finding will be discussed later. The individual results are further analysed in Figures 6 to 11, where they are compared to the range of $\pm$ one S.D. of the normal adult mean for rectus abdominis.

Figures 6 and 7 show respectively the total and extracellular water content of the biopsy-specimens. There is a tendency to an increase in both compared to the adult values, being most marked in the younger subjects and less obvious as age increases.

Figures 8 and 9 show the total sodium and chloride contents, which are again initially high, but tend to fall into the adult range around 18 months to 2 years.

Figure 10 shows the biopsy-specimen intracellular water, and Figure 11 the total potassium contents. There is an increase above the adult range in both in the youngest infants, but after about 6 months both approximate closely to the normal adult range.

There is a highly significant correlation between the extracellular water and the sodium contents (Figure 12) and between the intracellular water and potassium contents (Figure 13)

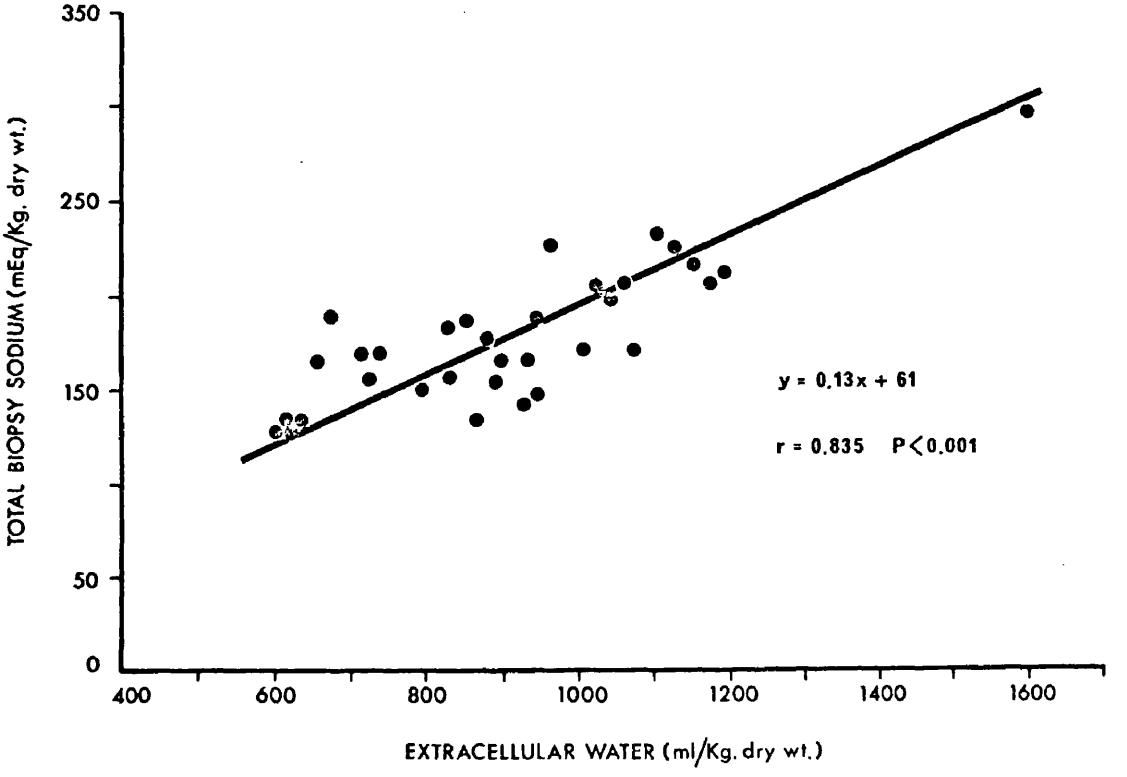

Figure 12: Correlation between biopsy-specimen extracellular water and total sodium content in 33 children.

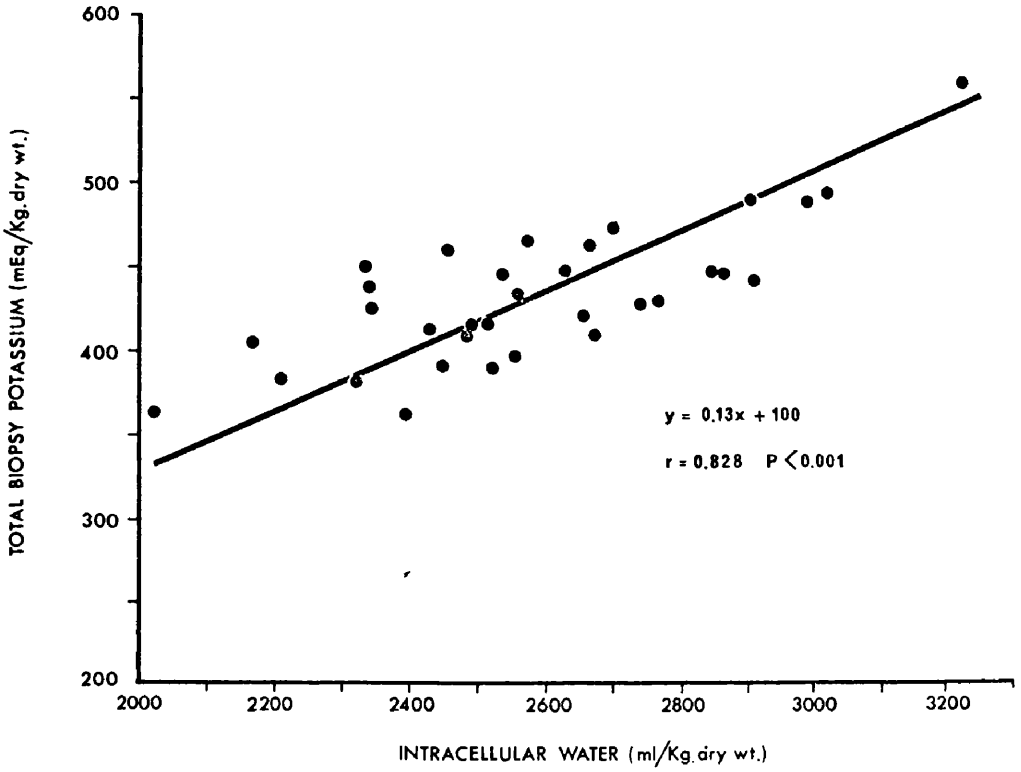

Figure 13: Correlation between biopsy-specimen intracellular water and total potassium content in 33 children.

throughout the entire age-range studied. These correlations have also been found in normal adults, and therefore appear to hold good at all ages. Because of these relationships the intracellular electrolyte concentrations lie within relatively narrow limits at all ages, and the mean values for all the subjects in this series are close to that of normal adults, being:- sodium, 20 $\pm$ 7 meq. per litre; potassium, 167 $\pm$ 13 meq. per litre; and chloride, 4.4 $\pm$ 0.2 meq. per litre.

DISCUSSION

In this study biopsy-specimens were only taken from children having elective surgical procedures, using muscles exposed by the surgical incision. This, however, raises some problems. It is difficult to find very young children who are having surgery and in whom it seems fairly certain that the body water and electrolytes have not been altered. Furthermore, these children may have had normal eating and drinking habits upset by admission to hospital. The high blood urea values in some of these cases were probably due to a degree of dehydration; McCance and Widdowson[19] have shown that the blood urea rises quickly in slightly dehydrated normal infants. Part of the variability of the results for water and electrolyte content in this and other series[1, 3, 20] may therefore be due to alterations in the intake of food or fluids.

One other possible cause of variation in these results may be that the biopsy-specimen results were taken from two different muscles, as it has been already shown (Chapter III) that different muscle groups have slightly different water and electrolyte contents. However, it has not been possible to compare results from the two muscles individually in this series, and they have therefore been grouped together; any error introduced by this is likely to be small.

The results, expressed per unit of body weight, show a similar trend to those of previous investigators. Extracellular water, and the extracellular ions sodium and chloride, are high in the newborn then fall slowly to reach adult values around 2 years of age. It has been shown[1, 9] that there is a further slow fall in the extracellular constituents after this age, and that adult values are not reached until adolescence. In this series, the group of children from 2 to 9 years had higher mean water and electrolyte values than the adult group, although only the differences in total water, and sodium and chloride contents are significant; this may be a reflection of a further slow decline towards adult values in these older children.

The results of this investigation suggest that intracellular water and potassium contents are high in the newborn, but fall quickly to adult values by about 6 months. Clapp, et. al.[5] have shown a similar fall in intracellular water in their subjects; and

Hazlewood, et. al.[21] showed a fall in potassium content with age in mouse skeletal muscle. However, Friis-Hansen[1] believed that intracellular water remained relatively steady throughout growth, and Forbes[7] and Corsa, et. al.[9] similarly found that the potassium content remained steady with growth; and other authors believe that cell potassium content increases with age[16, 17, 20]. Their results are all very variable, however, and they are expressed per wet weight of muscle tissue, which tends to obscure changes in electrolyte content and may explain this discrepancy.

An excellent correlation has been found between intracellular water and potassium contents in this series. This means that the intracellular potassium concentration lies within narrow limits at all the ages studied, the value being similar to that of adults. Similar findings have been reported in rats[22] and humans[9, 16, 17]. Despite changes in water and electrolytes during growth, the intracellular concentrations of electrolytes remain steady; this is vital for maintenance of normal electrical activity and therefore function in the cell. This directly contradicts the findings of Nichols, et. al.[20] that the intracellular potassium concentration was low at birth and increased with age. No apparent reason for this discrepancy in their results can be found.

Because of the variation in water and electrolyte contents in children of the same age, it is difficult to evaluate "normality"

at any age. However, muscle biopsy-specimens are easy to take and analyse, either at open operation or by percutaneous needle biopsy, and the results obtained will help to provide a more accurate picture of the fluid and electrolyte status of individual children. No results from ill children will be presented in this thesis, although data are being collected in a variety of pathological conditions at the present time.

SUMMARY

Extracellular water, sodium and chloride contents per dry weight of muscle tissue are high in infancy but fall towards adult values by 18 months to 2 years. There may be a further slow fall after this, adult values not being reached until adolescence.

Intracellular water and potassium contents are initially high, but approximate to adult values by 6 months of age.

There are good correlations between extracellular water and sodium contents, and intracellular water and potassium contents, so that intracellular electrolyte concentrations are similar for children of all ages, and are similar to adult values.

Due to variations in electrolyte content in children of the same age, it is difficult to evaluate "normality", but useful information can be obtained by this method about fluid and electrolyte balance in individual children.

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CHAPTER V

WATER AND ELECTROLYTE IMBALANCE IN PYLORIC STENOSIS

INTRODUCTION

The loss of gastric juice by vomiting or by accumulation in the dilated stomach in pyloric stenosis leads to a hypochloraemic alkalosis. Sodium and potassium are lost in the vomitus, and also in the urine as a result of the renal response to alkalosis.

Gamble and Ross[1] first stressed the importance of sodium loss in causing dehydration in pyloric stenosis. Kassirer and Schwartz[2-4] showed that chloride replacement was necessary to correct the alkalosis in pyloric stenosis. Burnett, et. al.[5] and Lans, et. al.[6] showed the necessity to replace potassium loss during treatment, but measurement of the potassium deficit by metabolic balance studies revealed variable losses[7-9].

There has been no direct measurement of the fluid and electrolyte losses in pyloric stenosis, and this chapter presents the changes found in skeletal muscle water and electrolytes before any replacement therapy. This work has been published by Graham[10].

PATIENTS AND METHODS

Eighteen patients with proven pyloric stenosis were divided into three groups on the basis of clinical findings and serum-electrolyte values, as follows:-

(1) Chronic Group: Regular vomiting for several months, but

TABLE XI

AGE, AETIOLOGY AND SERUM ELECTROLYTE VALUES IN THE THREE GROUPS OF PATIENTS WITH PYLORIC STENOSIS

NO.	AGE	AETIOLOGY	SERUM ELECTROLYTES (meq./l. or mg./100 ml.)				
			SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA
CHRONIC							
1	68	D.U.	137	4.0	96	27	32
2	64	D.U.	137	3.3	99	28	24
3	68	D.U.	140	4.9	100	29	52
4	50	CARCINOMA	138	5.0	94	29	89
5	60	D.U.	140	3.9	100	30	64
6	63	D.U.	141	4.6	98	30.5	61
SUBACUTE							
7	78	CARCINOMA	140	4.5	100	25	30
8	70	D.U.	141	5.0	98	25	40
9	74	CARCINOMA	138	3.2	75	32	84
10	44	D.U.	141	3.8	97	32.5	46
11	55	D.U.	139	3.1	89	32.5	52
12	70	D.U.	145	3.0	83	39	70
ACUTE							
13	57	D.U.	132	3.3	75	35.5	104
14	41	D.U.	130	3.6	71	35.5	61
15	71	D.U.	134	3.6	83	40	96
16	51	D.U.	130	3.1	78	42	80
17	70	D.U.	133	4.1	78	44	112
18	48	CARCINOMA	132	3.0	75	44	282

D.U. = chronic duodenal ulcer

TABLE XII

MEAN SERUM-ELECTROLYTE VALUES IN THE THREE GROUPS

OF PATIENTS WITH PYLORIC STENOSIS

GROUP		SERUM ELECTROLYTE CONCENTRATIONS meq. per litre				mg. per 100 ml.
		SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA
CHRONIC	MEAN	139	4.3	98	29	54
	S.D.	2	0.7	2	1	24
SUBACUTE	MEAN	141	3.8	90	31	54
	S.D.	2	0.8	10	5	20
ACUTE	MEAN	132	3.5	77	40	123
	S.D.	2	0.4	4	4	80

often only once daily. Serum-electrolyte concentrations were little altered.

(2) Subacute Group: More marked symptoms, but no signs of dehydration, and not clinically particularly ill. Hypochloraemic alkalosis was present, and the serum-potassium concentration was usually low.

(3) Acute Group: Severe vomiting, with dehydration, muscle weakness, mental confusion or even semicoma. Serum-electrolytes were grossly altered, alkalosis was severe, and all required immediate resuscitative treatment. Only 2 patients had chronic symptoms before the acute episode; in the others vomiting had been present for 2 - 8 weeks at most.

The results have been compared to the group of vastus lateralis biopsy-specimens taken from 42 normal subjects.

Biopsy-specimens were taken from vastus lateralis in each case, and analysed and partitioned as described in Chapter II.

RESULTS

Table Xl shows the age, aetiology of the stenosis, and the serum-electrolyte concentrations in the individual patients, sub-divided into the three groups. Table XII shows the mean serum-electrolyte values in the three groups. The normal ranges

TABLE XIV

MEAN BIOPSY-SPECIMEN RESULTS IN NORMAL SUBJECTS COMPARED WITH

MEAN RESULTS OF THE THREE GROUPS OF PATIENTS WITH PYLORIC STENOSIS

GROUP	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMAL SUBJECTS:									
MEAN	3187	703	2483	136	429	82	15	172	4.3
S.D.	168	135	180	27	32	14	7	8	0.2
CHRONIC P.S:									
MEAN	3291	940	2342	158	399	105	11	171	4.1
S.D.	237	110	176	10	22	12	4	10	0.1
SUBACUTE P.S:									
MEAN	3278	754	2520	145	425	78	15	168	3.8
S.D.	161	121	109	33	29	13	8	14	0.4
ACUTE P.S:									
MEAN	3101	457	2647	85	431	43	10	163	3.2
S.D.	101	89	106	9	24	6	5	11	0.1

TABLE XV

STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN NORMAL SUBJECTS

AND THREE GROUPS WITH PYLORIC STENOSIS, AND BETWEEN GROUPS THEMSELVES

	BIOPSY-SPECIMEN WATER			TOTAL BIOPSY-SPECIMEN ELECTROLYTES			INTRACELLULAR ELECTROLYTES		
GROUPS UNDER TEST	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMAL v. CHRONIC	N.S.	$P<0.001$	N.S.	N.S.	$P<0.05$	$P<0.001$	N.S.	N.S.	N.S.
NORMAL v. SUBACUTE	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	$P<0.001$
NORMAL v. ACUTE	N.S.	$P<0.001$	$P<0.05$	$P<0.001$	N.S.	$P<0.001$	N.S.	$P<0.02$	$P<0.001$
CHRONIC v. SUBACUTE	N.S.	$P<0.02$	N.S.	N.S.	N.S.	$P<0.01$	N.S.	N.S.	N.S.
CHRONIC v. ACUTE	N.S.	$P<0.001$	$P<0.01$	$P<0.001$	$P<0.05$	$P<0.001$	N.S.	N.S.	$P<0.001$
SUBACUTE v. ACUTE	$P<0.05$	$P<0.001$	N.S.	$P<0.01$	N.S.	$P<0.001$	N.S.	N.S.	$P<0.01$

are taken as:- sodium, 135 - 148 meq. per litre; potassium, 4.1 - 5.5 meq. per litre; chloride, 96 - 106 meq. per litre; bicarbonate, 24 - 31 meq. per litre. Hypochloraemic alkalosis was not seen in any of the chronic patients, but became more obvious as the symptoms of pyloric stenosis became more acute. Twelve patients had low serum potassium concentrations, again more common as the alkalosis became more severe. Only the patients with acute pyloric stenosis had significant hyponatraemia.

Table XIII gives the individual biopsy-specimen water and electrolytes for the three groups of patients, and Table XIV compares the mean results for each group to the mean values for the normal subjects. The statistical significance of the differences between each group is shown in Table XV. The findings may be summarised thus:-

(1) Chronic Group: The mean extracellular water and chloride contents were significantly greater than normal. The potassium content was lower than normal.

(2) Subacute Group: Individual biopsy-specimen results and the mean values were essentially normal. The intracellular chloride concentration was reduced (this being of course a reflection of the lower extracellular chloride concentration).

The chronic and subacute groups differed from each other only in the extracellular water and chloride contents, but both

TABLE XVI

FLUID BALANCE, ELECTROLYTE INTAKE AND CHANGES IN SERUM ELECTROLYTE CONCENTRATIONS AFTER RESUSCITATION IN TWO PATIENTS WITH ACUTE PYLORIC STENOSIS (FLUID OUTPUT COMPRISES URINE AND GASTRIC LOSSES AND 500 ml./DAY INSENSIBLE LOSS)

PATIENT	(Kg.) WEIGHT	FLUID BALANCE (ml.)		ELECTROLYTE INTAKE (meq.)			SERUM ELECTROLYTES (meq./l. or mg./100 ml.)				
		INTAKE	OUTPUT	SODIUM	CHLORIDE	POTASSIUM	SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA
No. 15											
Biopsy (1)	60.6						134	3.6	83	40	96
Biopsy (2)	62.1	6050	4460	675	755	80	135	5.2	98	23	71
(40 hours later)											
No. 18											
Biopsy (1)	37.3						132	3.0	75	44	282
Biopsy (2)	40.0	10290	7750	825	1025	200	133	4.2	94	29	92
(65 hours later)											

TABLE XVII

BIOPSY-SPECIMEN RESULTS BEFORE AND AFTER RESUSCITATION

IN TWO PATIENTS WITH ACUTE PYLORIC STENOSIS

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
No. 15									
Biopsy (1)	3132	442	2689	74	413	45	5	153	3.0
Biopsy (2)	3113	686	2427	114	417	77	10	170	4.1
No. 18									
Biopsy (1)	2909	392	2517	88	392	37	14	157	3.2
Biopsy (2)	3043	914	2128	137	390	95	9	181	3.9

had greatly different water and electrolyte contents from the acute group.

(3) Acute Group: Despite the clinical evidence of dehydration, the total water content was in the normal range in each case. However, as the mean extracellular water was significantly reduced and the intracellular water significantly raised, there appears to be a shift of water into the cell. The mean sodium and chloride contents are very low, but the potassium content was normal. As a result of the increased intracellular water, the intracellular potassium and chloride concentrations were significantly reduced.

In order to investigate further the unexpected finding of normal total water contents in these patients, repeat biopsies were carried out after resuscitation in two cases (numbers 15 and 18). The weight changes, details of the fluid balance and electrolyte intake, and serum-electrolyte values at the time of each biopsy are shown in Table XVI, and the results of the two biopsy-specimens are shown in Table XVII. The fluid balance and weight changes of the two patients agreed closely, and thus the actual fluid deficits were only of the order of 1.5 and 2.7 litres respectively. Electrolyte losses were not measured, but the total biopsy-specimen sodium and chloride contents were virtually normal after treatment, while the potassium content was unchanged.

The total water contents were very little altered, as would be expected from the small net gain of water, but the extracellular and intracellular fractions were restored to normal after resuscitation. Owing to the reduction in intracellular water, the intracellular potassium concentrations were also normal.

DISCUSSION

The findings of this series show that the metabolic upsets of pyloric stenosis are of two types. In patients with chronic vomiting, the tendency towards increase in extracellular water and electrolytes at the expense of the intracellular constituents resembles the changes found in "starvation"[11].

The biopsy-specimen results were essentially normal in the subacute group of patients. Some of these patients had long-standing chronic symptoms, and may well have had changes as described above, but then owing to increased vomiting the excess extracellular fluid and electrolytes were lost, giving the semblance of "normality". On the other hand, some of the patients had relatively recent onset of symptoms, which although severe enough to bring the patient to seek treatment, seems to have produced little metabolic upset.

In acute pyloric stenosis the mean biopsy-specimen sodium and chloride contents were reduced by 42% and 51% respectively, but the potassium content was normal. Owing to the loss of sodium and

chloride ions, the extracellular osmotic pressure falls; but the intracellular osmotic pressure is likely to be normal. Therefore an osmotic gradient will develop across the cell membrane, and the observed shift of water into the cell is necessary to restore osmotic equilibrium. An exactly similar picture to this has been described by Schloerb and Grantham[12] in the study of experimental pyloric stenosis in dogs.

The evidence of the biopsy-specimens together with the simple balance study suggests that the intracellular water shift is most important in the reduction of the extracellular space, and that the actual fluid loss is relatively small. Ghose[13] described a patient with acute pyloric stenosis and noted that the weight gain during resuscitation was only 0.5 Kg. Apparently these patients lose electrolytes in the vomitus well in excess of water. Despite the fact that gastric fluid is nearly always hypotonic to plasma[6, 9] considerable water absorption can take place from the stomach[14] and, as the patients drink between bouts of vomiting, it is quite possible that enough water may be absorbed from the stomach to keep the body water relatively normal. However, the electrolytes of the gastric juice are only very slowly reabsorbed and will be mostly lost in the vomitus.

The mean biopsy-specimen potassium contents were significantly lower than normal only in the patients with chronic pyloric stenosis,

and this tends to confirm the findings of Howe and Le Quesne[9] that potassium losses are not severe in pyloric stenosis. Two patients, however, had moderately low potassium contents (numbers 4 and 12, Table XIII), the actual body deficits being of the order of 450 and 350 meq. respectively. Sodium and chloride contents were normal in these cases, and they correspond to a similar case described by Black and Jepson[7] where the potassium loss was 800 meq. although sodium and chloride deficits were very small - the picture of chronic pyloric stenosis found in this series.

Four of the acutely ill patients had biopsy-specimen potassium contents greater than the normal mean. These patients had short histories of vomiting, and were all very alkalotic; it has been shown that alkalosis causes a shift of potassium into the cell[15, 16]; and this intracellular shift will tend to maintain body stores. However, owing to the increased intracellular water, the cell potassium concentration tends to be low in acute pyloric stenosis, and this may explain the muscle weakness commonly seen in these patients. The patients with chronic pyloric stenosis have a longer history of vomiting, and tend to be less alkalotic, and the impression gained from this study is that the more chronic the symptoms the greater the potassium deficit.

Finally it is known that large infusions of saline increase renal potassium excretion[17]. Despite parenteral potassium therapy,

the potassium content of the repeat biopsy-specimens in the two acute patients was virtually unaltered, suggesting that renal potassium excretion was high during the resuscitation period. It can be concluded therefore, that in most cases of pyloric stenosis it will only be necessary to replace potassium loss occurring during treatment, but potassium should be given in greater amounts where the chronicity of the history suggests the likelihood of a significant deficit.

SUMMARY

The water and electrolyte content of skeletal muscle biopsy-specimens taken from 18 patients with pyloric stenosis have been compared to those of normal subjects. The patients were divided into three groups on the basis of clinical findings and serum-electrolyte abnormalities.

In the chronic and subacute groups the changes were usually slight. Extracellular water and electrolytes tended to be increased, and intracellular water and potassium to be reduced; these findings are typical of "starvation".

In the acutely ill patients the extracellular water was low, but the intracellular water was high. Sodium and chloride contents were grossly diminished, but the potassium content was normal.

The shift of water into the cell appears to be due to the osmotic gradient across the cell membrane caused by loss of extracellular sodium and chloride. Repeat biopsies in two of these patients after resuscitation showed restoration of normality, and balance studies confirmed that the actual fluid deficit was much less than the clinical evidence suggested.

Only patients with a long history of chronic symptoms are likely to have significant potassium deficits. In acute pyloric stenosis the potassium loss is small, but potassium supplements should be given to replace urinary losses during resuscitation.

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CHAPTER VI

MUSCLE WATER AND ELECTROLYTES AFTER URETEROCOLIC ANASTOMOSIS

INTRODUCTION

Ferris and Odel[1] were first to describe the syndrome of hyperchloraemic acidosis following ureterocolic anastomosis, and the aetiology of this condition is now well understood[2-5]. Severe potassium deficiency was described in patients with hyperchloraemic acidosis soon after[6, 7], and Wilkinson[8] believed that most of the symptoms of hyperchloraemic acidosis were due to potassium loss.

Despite this early evidence for potassium depletion, there are only two published accounts on total body potassium changes after ureterocolic anastomosis[9, 10] which give slightly different findings. This study describes the changes found in skeletal muscle water and electrolytes in patients with hyperchloraemic acidosis of varying severity. The work presented here is to be published[11].

PATIENTS AND METHODS

Eight biopsy-specimens have been analysed from 6 patients with hyperchloraemic acidosis. The results have been divided into two groups, (i) 3 patients with severe acute symptoms, and (ii) 5 patients with well controlled symptoms (2 patients having repeat biopsies after recovery from their acute episodes).

The results are compared to those of the vastus lateralis biopsy-specimens taken from 42 normal subjects.

TABLE XVIII

SERUM ELECTROLYTE VALUES AND BIOPSY-SPECIMEN RESULTS OF THREE PATIENTS WITH SEVERE HYPERCHLORAEMIC ACIDOSIS

	SERUM ELECTROLYTES (meq. per litre or mg. per 100 ml.)					BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
PATIENT	SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
G.F.	149	2.8	116	9	97	2896	934	1962	184	264	115	22	134	4.8
J.McP.	148	2.9	122	10	131	2895	857	2038	190	361	115	32	177	5.1
M.W.	141	3.4	102	16	260	2856	672	2184	98	377	78	4	172	4.3

TABLE XIX

SERUM ELECTROLYTE VALUES AND BIOPSY-SPECIMEN RESULTS OF FIVE PATIENTS WITH WELL CONTROLLED HYPERCHLORAEMIC ACIDOSIS

	SERUM ELECTROLYTES (meq. per litre or mg. per 100 ml.)					BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
PATIENT	SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
J. McG.	144	3.2	118	15.5	63	3485	1042	2443	149	384	135	5	156	4.9
G.F.	138	4.0	114	15	70	3450	829	2621	204	395	107	34	150	4.7
C.D.	148	4.0	105	23.5	214	3034	882	2151	187	401	102	26	185	4.4
J.McP.	143	3.3	111	21	98	3667	974	2693	159	427	121	7	157	4.6
J.B.	143	3.8	118	15	92	3884	1037	2846	184	451	137	13	157	4.9

TABLE XX

MEAN BIOPSY-SPECIMEN VALUES FOR NORMAL SUBJECTS COMPARED TO THE MEAN VALUES OF PATIENTS WITH SEVERE OR WELL-CONTROLLED HYPERCHLORAEMIC ACIDOSIS

GROUP	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLUIAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMALS									
MEAN	3187	703	2483	136	429	82	15	172	4.3
S.D.	168	135	180	27	32	14	7	8	0.2
SEVERE H.A.									
MEAN	2882	821	2061	157	334	103	19	161	4.7
S.D.	23	135	113	51	61	21	14	24	0.4
CONTROLLED H.A.									
MEAN	3504	953	2551	177	412	120	17	161	4.7
S.D.	314	95	266	22	27	16	12	14	0.2

H.A. = Hyperchloraemic Acidosis

TABLE XXI

STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN NORMAL SUBJECTS AND GROUPS OF PATIENTS WITH HYPERCHLORAEMIC ACIDOSIS

	BIOPSY-SPECIMEN WATER			TOTAL BIOPSY-SPECIMEN ELECTROLYTES			INTRACELLULAR ELECTROLYTES		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMAL v. SEVERE	$P<0.005$	N.S.	$P<0.001$	N.S.	$P<0.001$	$P<0.02$	N.S.	N.S.	$P<0.001$
NORMAL v. CONTROLLED	$P<0.001$	$P<0.001$	N.S.	$P<0.005$	N.S.	$P<0.001$	N.S.	$P<0.01$	$P<0.001$

N.S. = not significant.

Biopsy-specimens were taken from the vastus lateralis in all cases, and analysed and partitioned as described in Chapter II.

RESULTS

The normal serum-electrolyte ranges are: sodium, 135 - 148 meq. per litre; potassium, 4.1 - 5.5 meq. per litre; chloride, 96 - 106 meq. per litre; bicarbonate, 24 - 31 meq. per litre.

Table XVIII shows the serum-electrolyte concentrations and the biopsy-specimen results of 3 patients with severe hyperchloraemic acidosis. Table XIX shows the same value for 5 patients with well controlled symptoms. The mean biopsy-specimen water and electrolyte contents in normal subjects are compared to the mean results of the two groups of patients with hyperchloraemic acidosis in Table XX, and the statistical significance of the differences from normal is shown in Table XXI. The essential findings may be summarised thus:-

1) Severe hyperchloraemic acidosis: The mean total and intracellular water contents are significantly lower than normal. Despite this dehydration, the extracellular water was in the normal range in each case. The total potassium content was significantly reduced. The total chloride content and the intracellular chloride concentration were both increased.

2) Well controlled hyperchloraemic acidosis: The mean total and extracellular water contents, and the total sodium and chloride

contents were all significantly higher than normal. The mean total potassium content is normal, but the intracellular potassium concentration is reduced. The intracellular chloride concentration is higher than normal.

As the clinical condition and therapeutic regime of each patient varied widely, brief clinical details are now given.

Patient G.F. Female, aged 43 years. Right nephrectomy in 1944 and left ureterocolic anastomosis in 1952 for urinary tuberculosis. She was well except for occasional urinary infections until sudden deterioration in 1967, and she was admitted to hospital moribund and with severe hyperchloraemic acidosis. Peritoneal dialysis was started, and muscle biopsy was taken 12 hours later (Table XVIII, number 1). This showed gross potassium deficiency and intracellular dehydration. She was given 700 meq. potassium in the next 24 hours and improved rapidly.

Three months later, when she was well and taking oral bicarbonate and potassium supplements, the results of a second biopsy (Table XIX, number 2) were all in the normal range except that the intracellular potassium concentration was still low.

She now has a colonic conduit and is in good health with only mild hyperchloraemic acidosis, controlled by oral bicarbonate supplements.

Patient J.McP. Male, aged 47 years. Right nephrectomy in 1940 and left ureterocolic anastomosis in 1949 for urinary tuberculosis. He had mild hyperchloraemic acidosis and a slowly rising blood urea for several years, but remained in good health. After a respiratory infection he became acutely ill with severe hyperchloraemic acidosis. Muscle biopsy again revealed potassium deficiency and intracellular dehydration (Table XVIII, number 2). After intravenous therapy with 400 meq. potassium in 36 hours, he had rapid clinical improvement, and was started on oral bicarbonate and potassium supplements.

A second biopsy 6 days later (Table XIX, number 4) showed an increased water content, and the potassium content was now quite normal. Balance study confirmed water retention of approximately 6 litres between the two biopsies.

At present he is well, but the blood urea remains around 90 mg. per 100 ml.

Patient M.W. Female, aged 66 years. Bilateral ureterocolic anastomosis in 1968 for intractable cystitis. She was well for 5 days post-operatively, then became dehydrated, oliguric and acidotic, although not hyperchloraemic. Muscle biopsy showed dehydration and low sodium, potassium and chloride contents (Table XVIII, number 3). The reduced sodium and chloride contents in this case were thought to be due to the development of acute renal failure combined with

some vomiting in the post-operative period.

She required several haemodialyses before renal function improved, and is at present fairly well with hyperchloraemic acidosis controlled by diet and oral bicarbonate supplements.

Patient J.McG. Male, aged 45 years. Total cystectomy and bilateral ureterocolic anastomosis for bladder carcinoma in 1963. He had recurrent urinary infections and chronic hyperchloraemic acidosis, fairly well controlled with oral bicarbonate supplements at the time of biopsy in 1967 (Table XIX, number 1). The extracellular water and chloride contents are high, and the potassium content and intracellular concentration are in the low normal range.

He continued in hyperchloraemic acidosis, with a rising blood urea and died in 1969 from uraemia and septicaemia.

Patient C.D. Male, aged 53 years. Bilateral ureterocolic anastomosis for interstitial cystitis in 1964. He had repeated urinary infections and several episodes of acute hyperchloraemic acidosis, with increasing renal impairment, and was admitted to hospital in 1967 to have a colonic conduit fashioned. After therapy with bicarbonate and potassium supplements, a muscle biopsy was taken (Table XIX, number 3), and showed the tendency to expansion of the extracellular contents seen in all these patients.

Despite further surgery he remained in renal failure and died 4 months later.

Patient J.B. Male, aged 58 years. Total cystectomy and bilateral ureterocolic anastomosis for bladder carcinoma in 1968. Since then he had frequent urinary infections with exacerbation of his hyperchloraemic acidosis, and at the time of biopsy one year later, had been treated with parenteral fluids, including 120 meq. potassium because of worsening acidosis. All the water values and the chloride content are high (Table XIX, number 5).

He is well at present, with well controlled hyperchloraemic acidosis.

DISCUSSION

Renal impairment is thought to be necessary for the development of hyperchloraemic acidosis after ureterocolic anastomosis[2, 12, 13]. All the patients in this series had persistently elevated blood urea concentrations, and the biopsy-specimen results showed an expansion of the extracellular water, sodium and chloride contents; this is the main finding in chronic renal failure, whether measured by muscle biopsy (see Chapter VII), or by isotope-dilution[14, 15]. The 3 acutely ill patients in this series all had marked intracellular dehydration. However, the extracellular water content was higher than the normal mean value in two of these patients. This explains the clinical impression that both these

patients were not dehydrated, as clinical evidence of hydration is a function of the extracellular water content of the body.

Severe potassium loss after ureterocolic anastomosis was first described by Foster, et. al.[6] and Diefenbach, et. al.[7], and Jacobs and Stirling[12] found hypokalaemia in 35% of the patients they studied. Wilkinson[8] and Pyrah[3] both stressed the likelihood of intracellular potassium depletion in patients with hyperchloraemic acidosis. Ansell and Creevy[9] measured total exchangeable potassium and found a mean reduction of 33% over normal controls; these patients were said to be normally active at the time of study. Williams, et. al.[10] repeated this work, but found a mean reduction of only 10% from the expected normal values. Some cases were deficient by up to 30%, but they noted that the total body potassium was greater than expected in 4 patients.

The results found in this study are also variable. In the acutely ill patients the muscle potassium content was obviously reduced, and the severity of the deficiency was related to the clinical symptoms. There is a close correlation between intracellular water and total potassium content, both in health (Chapter III) and in chronic renal failure (Chapter VI), so that the intracellular potassium concentration lies within narrow limits. The intracellular dehydration found in the acutely ill patients is probably therefore a response to the potassium loss, and the intracellular potassium

concentration has remained within normal limits in 2 of the patients; the potassium loss in the first patient was so gross, however, that the intracellular potassium concentration has fallen, and this may explain the severity of this patient's illness. It was estimated that the total potassium deficit in these patients was of the order of 1100, 600 and 400 meq. respectively.

The patients with well controlled hyperchloraemic acidosis show a greater variation in potassium content. The first 2 patients in Table XIX were both maintained on oral therapy, only the second getting potassium supplements, and the potassium contents were in the low normal range. The next 2 patients had both had intravenous potassium therapy and had normal potassium contents. However, the last patient, who had poorly controlled hyperchloraemic acidosis for several months, and who had received very little potassium, had a high normal potassium content.

There is no doubt therefore that the findings of this series support the conclusions of Williams, et. al.[10] that in acutely ill patients with hyperchloraemic acidosis large amounts of potassium are required to correct the body deficit, in addition to other therapeutic measures. The position regarding long-term oral therapy is, however, less clear. Most of these patients probably have some reduction of body potassium stores, and therefore continuous replacement of the increased urinary losses known to occur would be

theoretically worthwhile, despite the again largely theoretical risk of hyperkalaemia due to renal impairment. There is no evidence in the literature to suggest that continuous potassium therapy prevents the onset of severe hyperchloraemic acidosis, but there is also no evidence that it has caused any harm.

SUMMARY

The fluid and electrolyte imbalance in hyperchloraemic acidosis following ureterocolic anastomosis has been measured using the muscle biopsy technique. Three acutely ill patients and 5 with well controlled symptoms have been studied (including repeat biopsy on 2 ill patients after recovery).

All the acutely ill patients had marked intracellular dehydration, but in all the cases there was a tendency to an expansion of the extracellular water, sodium and chloride contents, the usual finding in chronic renal failure.

Muscle potassium contents were reduced in the ill patients, but varied in the well controlled patients from low to high normal values.

Large amounts of potassium are required in the treatment of patients severely ill with hyperchloraemic acidosis, but the value of long-term potassium therapy in well controlled cases is doubtful.

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CHAPTER VII

THE METABOLIC IMBALANCE OF CHRONIC RENAL FAILURE

INTRODUCTION

The water and electrolyte status of patients with varying degrees of chronic renal failure is studied in this chapter, excluding only patients on regular dialysis treatment. Previous studies have suggested that most patients with chronic renal failure show an increase in extracellular water and sodium, measured both by isotope-dilution[1-3] and by muscle biopsy analysis[4-7]; the effect of renal impairment on intracellular water and potassium is more difficult to evaluate. Moore, et. al.[1] found low total exchangeable potassium using isotope-dilution techniques, but this may in part be due to loss of lean body mass in these patients. Using muscle biopsy analysis, Villamil, et. al.[5] and Campanacci, et. al.[6] found low cell potassium contents, while Bergström and Hultman[7] found the reverse. Bittar, et. al.[4] obtained variable results.

This study attempts to elucidate these previous findings by relating changes in biopsy-specimen water and electrolytes to the degree of renal failure. Also the relationship between water and electrolyte contents and blood pressure is examined. The findings have been reported by Graham, et. al.[8, 9].

TABLE XXII

SERUM ELECTROLYTE VALUES, CREATININE CLEARANCE AND DIASTOLIC BLOOD-PRESSURE OF THE PATIENTS WITH CHRONIC RENAL FAILURE

PATIENT	SERUM ELECTROLYTE CONCENTRATIONS (meq. per litre)				(mg. per 100 ml.)		CREATININE CLEARANCE (ml. per min.)	DIASTOLIC BLOOD-PRESSURE (mm. Hg.)
	SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA	CREATININE		
1	144	4.7	105	18	56	2.5	20	80
2	144	4.6	117	13.5	53	3.3	17	80
3	140	5.2	109	20	90	3.4	13	115
4	140	4.7	106	21	112	4.0	15	130
5	148	4.6	119	15.5	144	5.2	11	70
6	146	6.1	115	18	88	5.3	-	120
7	140	6.4	106	20	115	5.8	4	110
8	140	4.7	113	16.5	144	6.1	14	80
9	144	4.7	112	18	93	6.6	6	70
10	135	3.3	92	20	245	8.1	7	80
11	137	4.9	94	23	170	8.1	3	140
12	139	4.8	113	15.5	128	8.3	7	110
13	141	4.2	106	17.5	94	9.0	5	75
14	134	4.3	97	9.0	209	9.0	2	95
15	146	4.1	110	15.5	184	9.0	4	85
16	124	5.9	93	9.5	184	-	-	110
17	138	4.5	103	15	226	9.3	6	90
18	143	4.4	116	10	229	10.4	4	80
19	143	4.5	87	35	204	11.2	7	80
20	142	6.6	117	14	147	12.6	7	110
21	131	4.8	100	13.5	240	13.1	4	110
22	131	6.4	100	8	230	16.3	3	100

PATIENTS AND METHODS

Twenty-two patients with chronic renal failure were studied. All had elevated serum creatinine concentrations (2.5 up to 16.3 mg. per 100 ml.), and endogenous creatinine clearances ranging from 3 ml. to 20 ml. per minute. All patients were taking a modified Giordano-Giovannetti diet providing either 18 G. or 40 G. of protein; the switch to low-protein diet being made when the serum creatinine concentration was greater than 8 mg. per 100 ml.

Two patients were receiving anti-hypertensive drugs and 8 diuretics at the time of the study; comparison of the biopsy-specimen results found in the patients receiving diuretics with the group not receiving such therapy did not reveal any significant differences, and all the results have therefore been examined together. Blood pressures were taken regularly under steady-state conditions.

The results have been compared to those of the vastus lateralis biopsy-specimens taken from 42 normal subjects.

Biopsy-specimens were taken from the vastus lateralis in each case and were analysed and partitioned as described in Chapter II.

RESULTS

Table XXII shows the serum-electrolyte concentrations,

TABLE XXIII

AVERAGE VALUES FOR ALL VASTUS LATERALIS BIOPSY-SPECIMENS

VALUE		BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
		TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
	MEAN	3187	703	2483	136	429	82	15	172	4.3
(n=42)	S.D.	168	135	180	27	32	14	7	8	0.2
95% CONFIDENCE LIMITS		2851-3523	433-973	2123-2843	82-190	365-493	54-110	1-29	156-188	3.9-4.7

TABLE XXIV

BIOPSY-SPECIMEN RESULTS OF INDIVIDUAL PATIENTS WITH CHRONIC RENAL FAILURE

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
1	3407	979	2427	163	438	114	9	179	4.4
2	3226	1140	2085	166	381	144	2	182	4.9
3	3338	1437	1901	221	388	165	10	197	4.5
4	3293	654	2638	114	424	81	8	160	4.4
5	3246	802	2444	230	435	108	46	176	5.0
6	3968	1418	2550	270	456	175	24	175	4.8
7	3362	961	2400	171	389	113	15	160	4.4
8	2970	1141	1828	187	349	138	17	187	4.7
9	2759	588	2171	130	420	76	21	193	4.7
10	3454	689	2764	126	492	74	12	177	3.8
11	4408	2028	2380	249	470	200	12	193	3.9
12	3381	1059	2321	191	407	131	21	173	4.7
13	3195	708	2487	181	458	86	33	183	4.4
14	3250	1205	2046	210	341	121	25	167	4.0
15	4012	1172	2839	212	413	142	14	144	4.6
16	3124	1344	1780	146	344	132	12	188	3.9
17	3327	905	2421	205	414	104	33	170	4.3
18	3665	877	2787	182	471	116	20	168	4.8
19	3333	1097	2235	191	388	104	15	172	3.6
20	3600	1248	2352	213	445	158	15	186	4.9
21	3961	1123	2838	182	465	124	12	162	4.2
22	4470	2179	2291	288	451	227	4	191	4.2

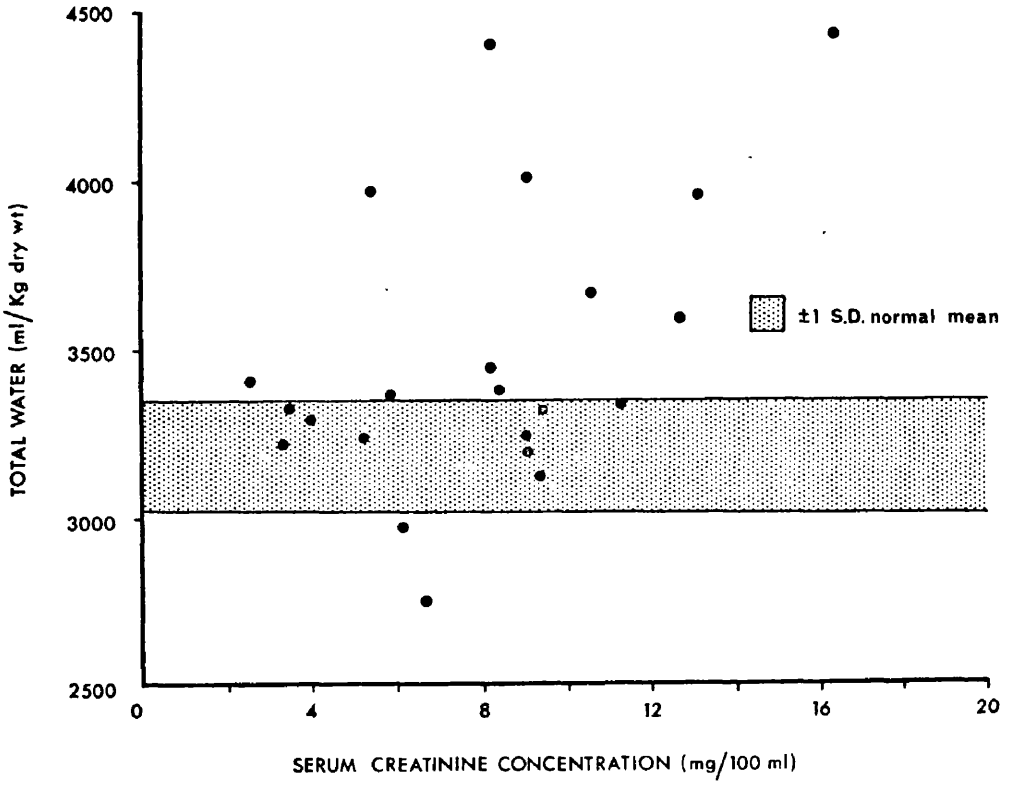

Figure 14: Biopsy-specimen total water as a function of serum creatinine concentration in 22 patients with chronic renal failure, compared to $\pm$ 1 S.D. normal acult mean.

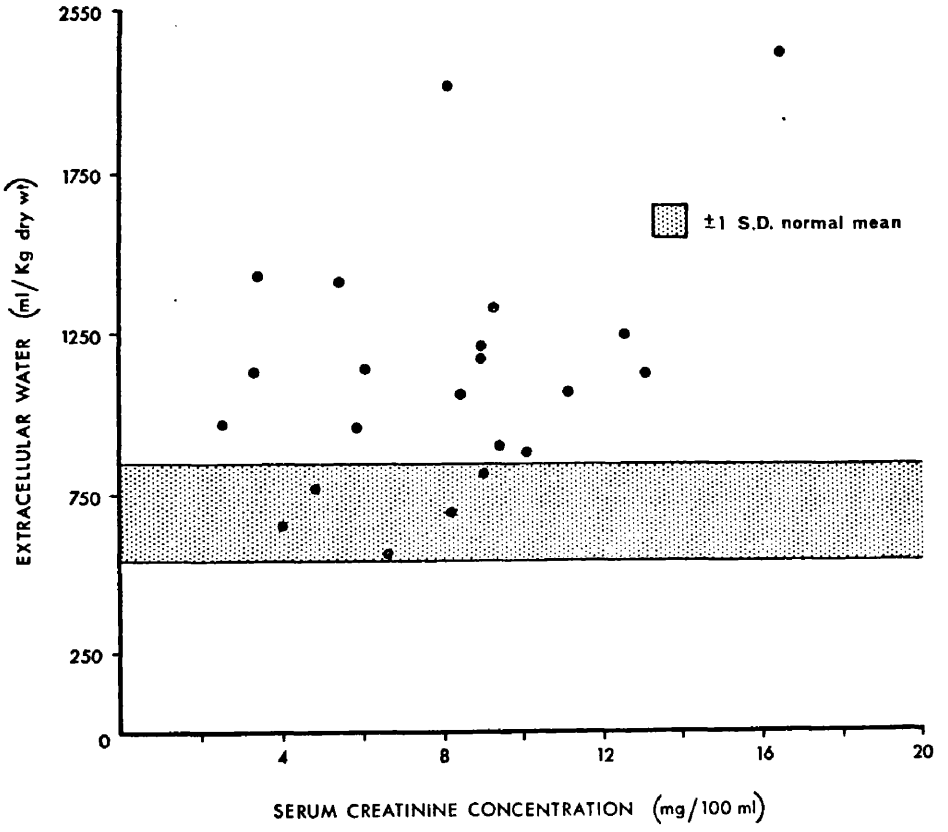

Figure 15: Biopsy-specimen extracellular water as a function of serum creatinine concentration in 22 patients with chronic renal failure, compared to $\pm$ 1 S.D. normal adult mean.

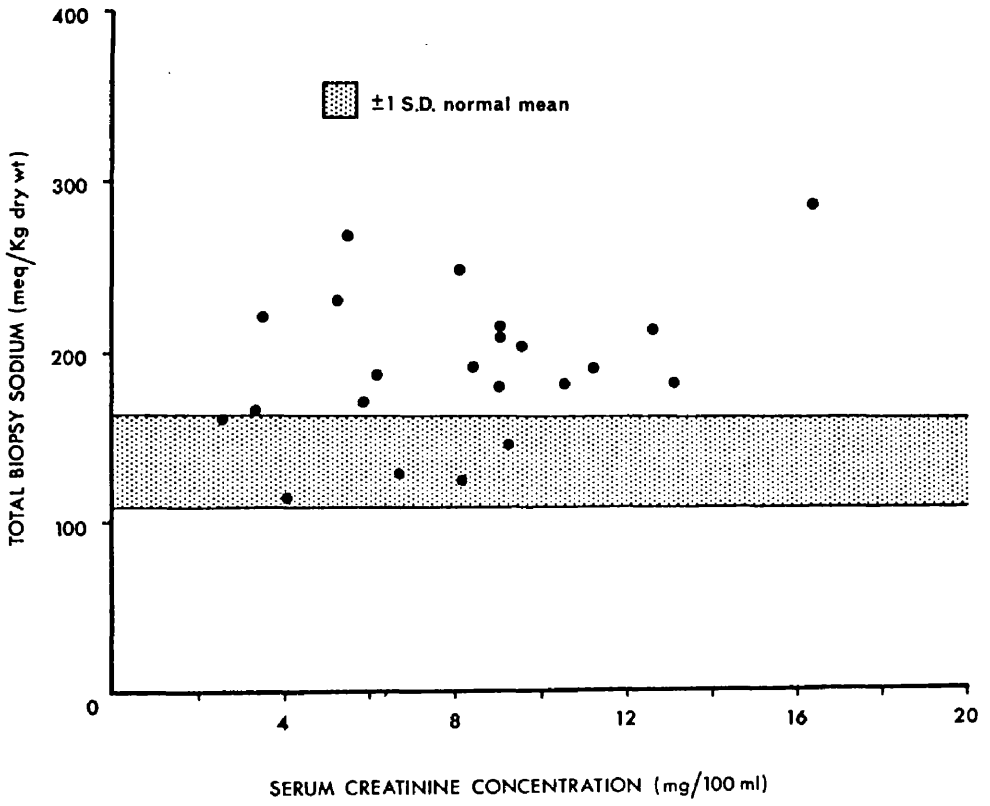

Figure 16: Total biopsy-specimen sodium content as a function of serum creatinine concentration in 22 patients with chronic renal failure, compared to $\pm$ 1 S.D. normal adult mean.

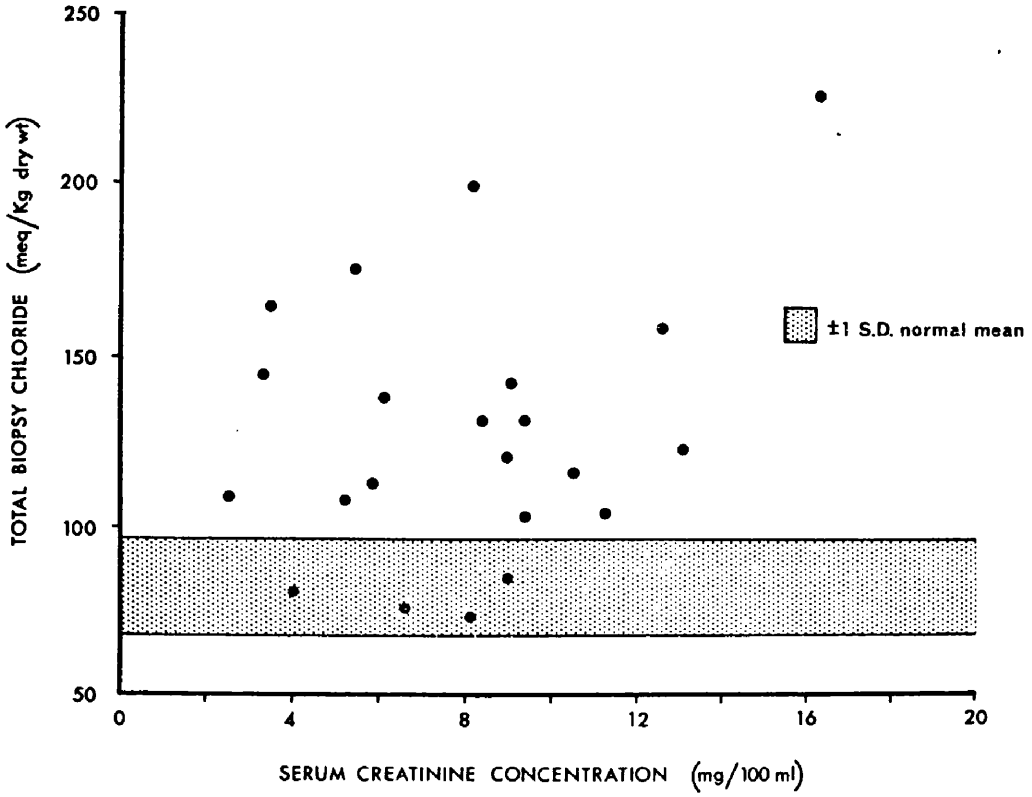

Figure 17: Total biopsy-specimen chloride content as a function of serum creatinine concentration in 22 patients with chronic renal failure, compared to $\pm$ 1 S.D. normal adult mean.

creatinine clearance and diastolic blood-pressure of the 22 patients with chronic renal failure. Normal serum-electrolyte ranges are:- sodium, 135 - 148 meq. per litre; potassium, 4.1 - 5.5 meq. per litre; chloride, 96 - 106 meq. per litre; bicarbonate, 24 - 31 meq. per litre.

Table XXIII shows again the mean values and the 95% confidence limits of the vastus lateralis biopsy-specimens from normal subjects. The biopsy-specimen findings in individual patients with chronic renal failure are listed in Table XXIV, and the values are further examined in Figures 14 - 19, in which they are compared to the range of $\pm$ 1 S.D. of the normal mean results.

Figures 14 and 15 show the total water and extracellular water contents respectively of the biopsy-specimens. There is a tendency towards an increase in both in chronic renal failure, becoming more common in the patients in the later stages of the disease. Total water is greater than the 95% confidence limits in 7 patients, and extracellular water is similarly increased in 14 patients. Total water is significantly reduced in only one patient. Figures 16 and 17 show the total sodium and chloride contents of the biopsy-specimens, and the tendency to an increase in both in chronic renal failure is clearly seen. The sodium content was significantly increased in 11 patients and the chloride content in 15. Despite this increase in sodium content, all patients had normal or low

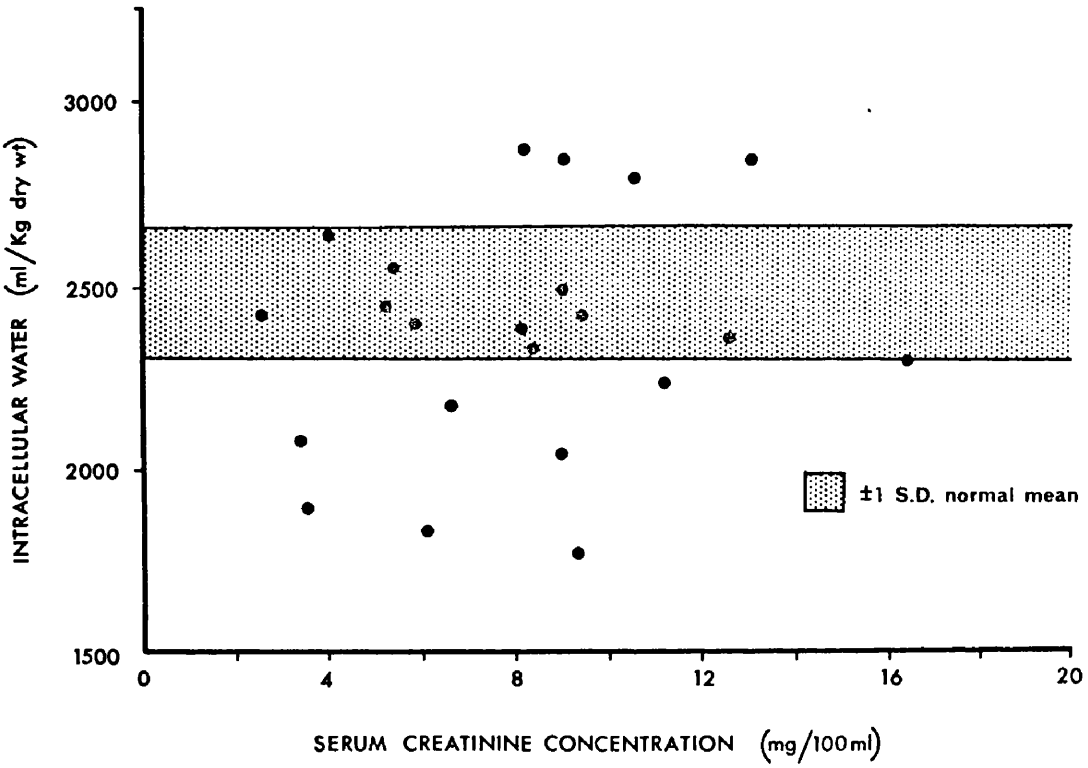

Figure 18: Biopsy-specimen intracellular water as a function of serum creatinine concentration in 22 patients with chronic renal failure, compared to $\pm$ 1 S.D. normal adult mean.

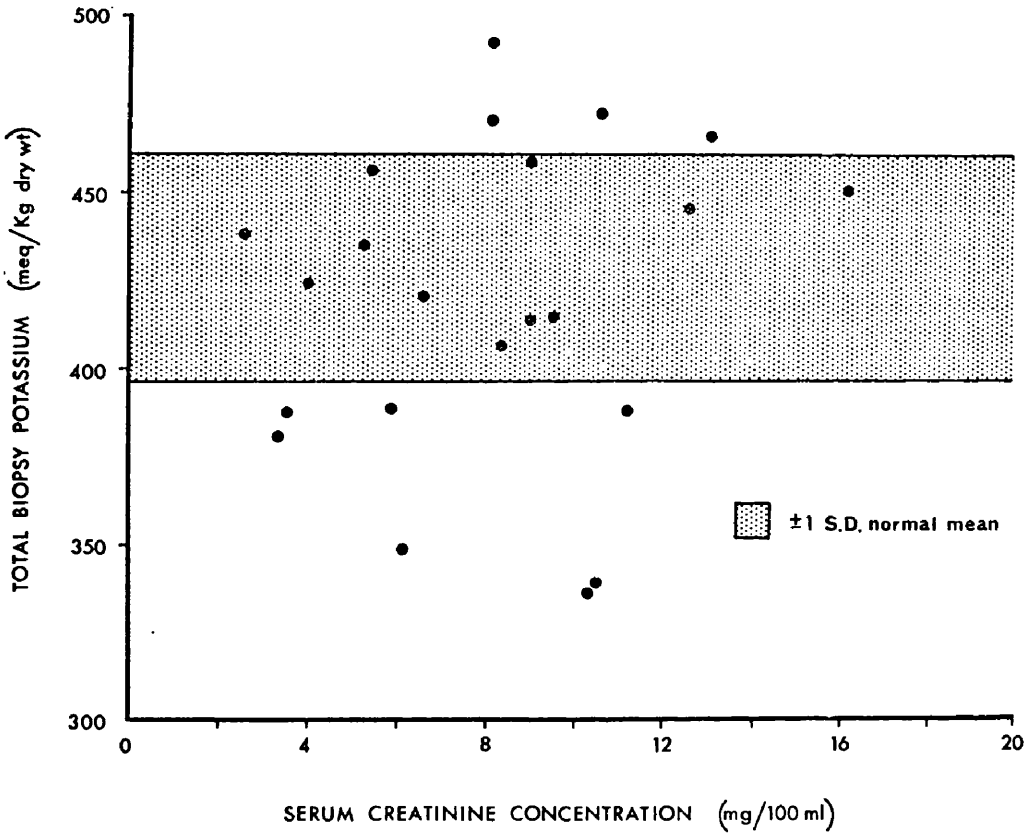

Figure 19: Total biopsy-specimen potassium content as a function of serum creatinine concentration in 22 patients with chronic renal failure, compared to $\pm$ 1 S.D. normal adult mean.

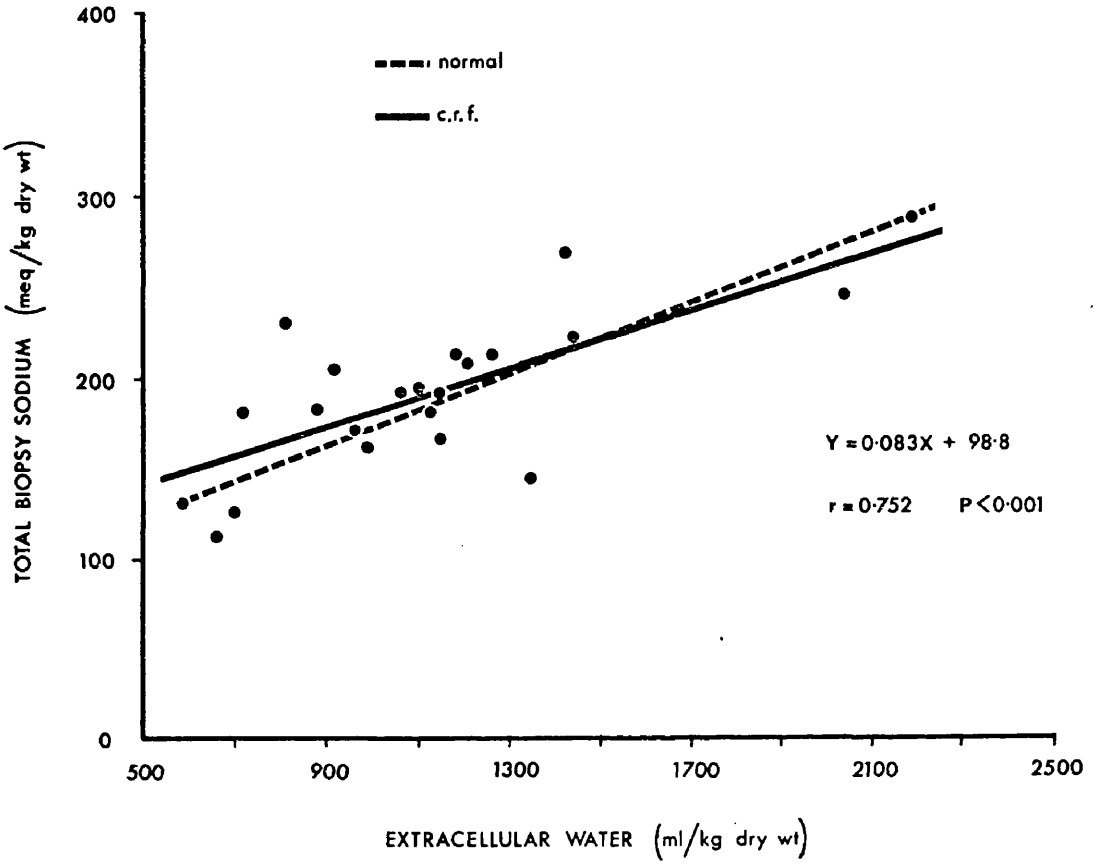

Figure 20: Correlation between biopsy-specimen extracellular water and total sodium content in 22 patients with chronic renal failure.

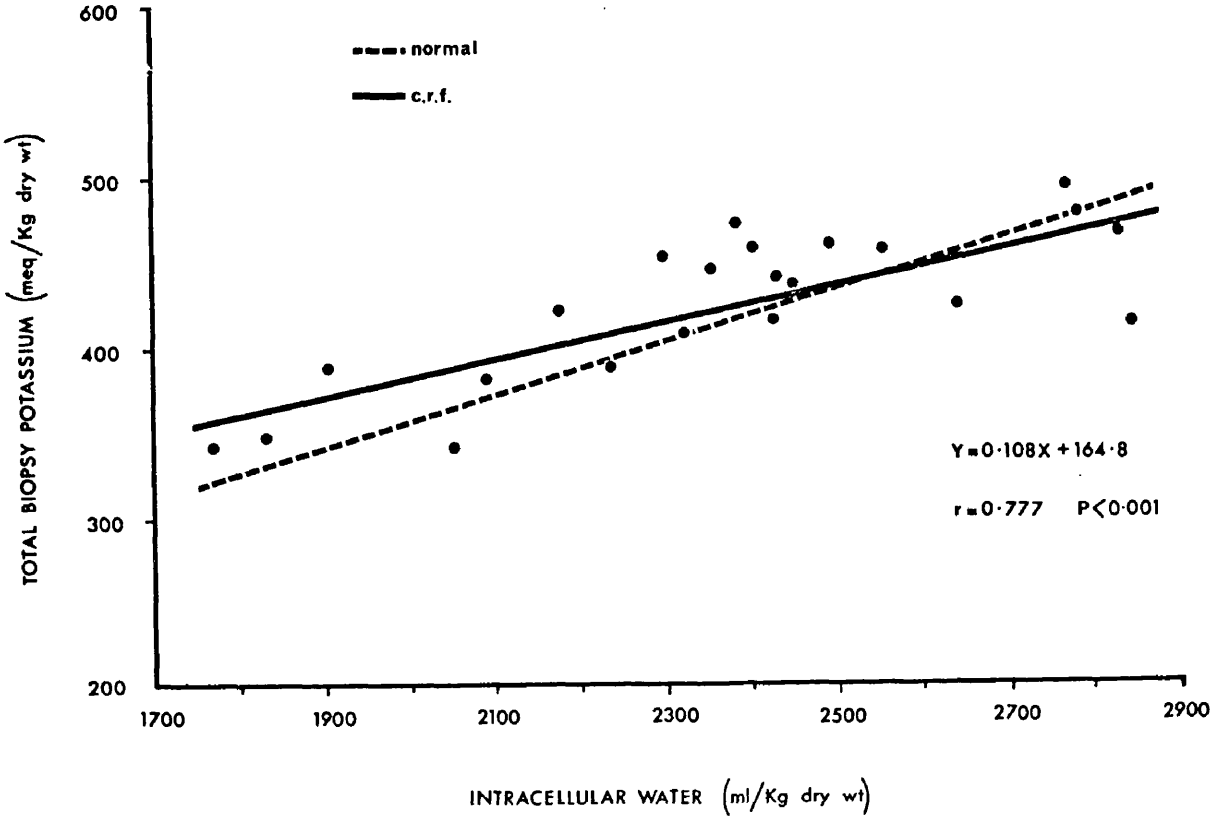

Figure 21: Correlation between biopsy-specimen intracellular water and total potassium content in 22 patients with chronic renal failure.

serum sodium concentrations, suggesting that extracellular water was increased to a greater degree than sodium.

On the other hand the intracellular water content tended to be within the normal range, or to be reduced (Figure 18); significant reduction being seen in 5 patients, and no patient had an intracellular water content above the normal 95% confidence limits. Figure 19 shows a similar trend in total potassium content, the values lying within normal limits in all but three patients who had significantly reduced contents. These 3 patients all had reduced intracellular water and therefore normal intracellular potassium concentrations; none were on diuretics.

Figures 20 and 21 demonstrate significant correlations between extracellular water and sodium content, and between intracellular water and potassium content in the patients with chronic renal failure. These relationships have already been shown in normal subjects (see Chapter III), and the regression lines for the normal series are not significantly different from those of the patients with chronic renal failure. Despite these correlations the intracellular potassium concentration was above the upper limit of normal in 5 cases, and significantly reduced in 1 patient; the intracellular sodium concentration was high in 3 cases.

A significant correlation was found between diastolic blood-pressure and the biopsy-specimen extracellular water content

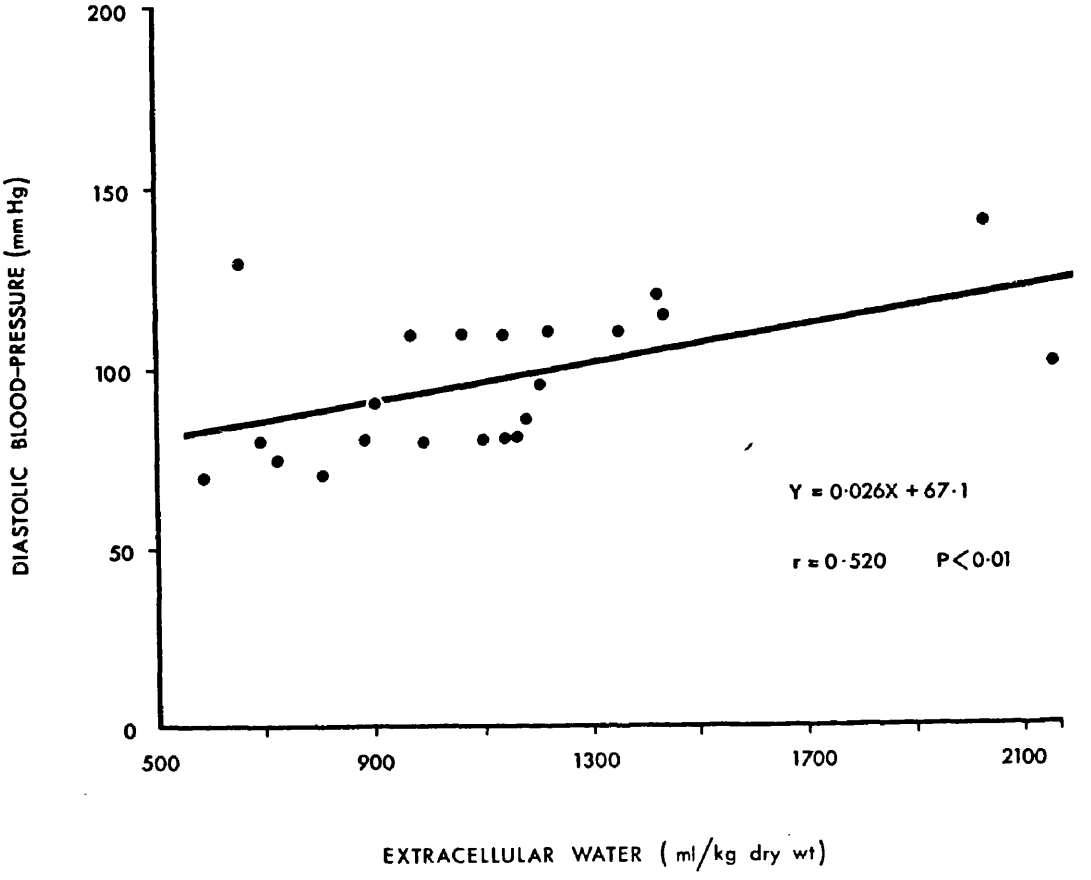

Figure 22: Correlation between biopsy-specimen extracellular water and diastolic blood-pressure in 22 patients with chronic renal failure.

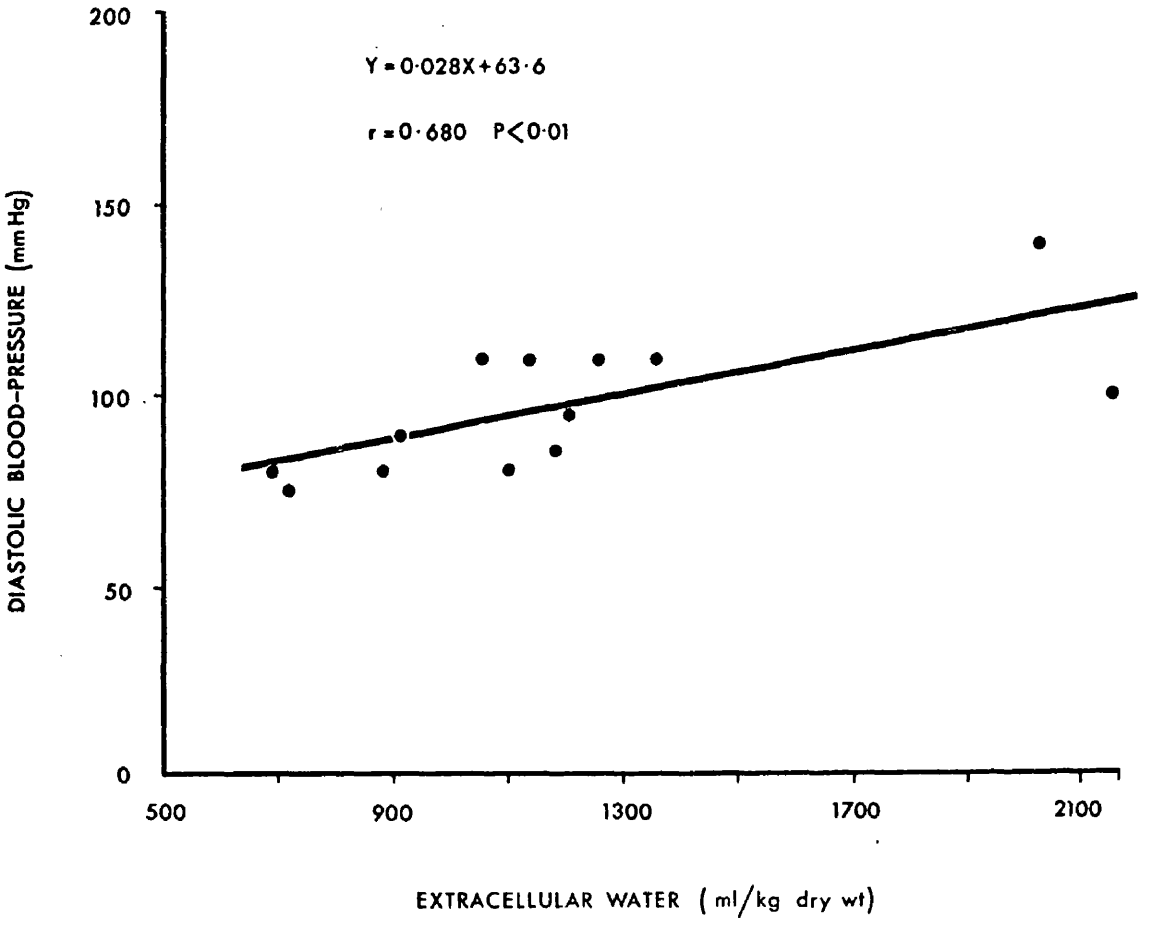

Figure 23: Correlation between biopsy-specimen extracellular water and diastolic blood pressure in 13 patients with severe chronic renal failure (serum creatinine concentration more than 8 mg. per 100 ml.).

(Figure 22), and this relationship became even more significant in the 13 patients with serum creatinine concentrations greater than 8 mg. per 100 ml. (Figure 23). No correlation could be found between diastolic blood-pressure and biopsy-specimen sodium content.

The mean serum sodium concentrations in the patients with diastolic blood-pressures greater than 90 mm. Hg. was 137 $\pm$ 6 meq. per litre ($\pm$ 1 S.D.) and those with diastolic blood-pressures less than 90 mm. Hg. was 143 $\pm$ 4 meq. per litre; this difference was statistically significant ($t = 2.78$, $p < 0.02$).

DISCUSSION

As discussed earlier, the calculation of the extracellular water of the biopsy-specimens will be accurate only if it can be assumed that the cell membrane potential does not vary by more than $\pm$ 10 mV from the arbitrary figure of -85 mV. The evidence suggesting that this is the case in normal subjects has already been given (Chapter II), together with some general evidence that changes in potential greater than 10 mV are unlikely in clinical practice. It is, however, possible that cell membrane potentials may be abnormal in uraemic subjects. Although it is well known that many biochemical systems can be altered by uraemic plasma *in vitro*, it is not known how many of these affect the patient's cells. With regard to membrane potential Bittar[10] has shown that injection of

uraemic plasma into the squid axon does not change the membrane potential. Furthermore, in uraemia, there is no significant change in intracellular pH[4, 11, 12, 13], nor on sodium flux across the jejunal wall[14]. Both these facts suggest that active transport systems are functioning normally in uraemia and such mechanisms are important in the maintenance of normal membrane potential.

Our results suggest that as chronic renal failure becomes more severe, there is increasing retention of water, sodium and chloride, resulting in an expansion of the extracellular space. Extracellular water retention tends to be greater than sodium retention, and thus serum sodium levels are usually normal or low. Intracellular water and electrolyte contents remain fairly normal except that in some cases there may be a reduction in cell potassium content, which is compensated for by an equivalent reduction in intracellular water, maintaining the intracellular potassium concentration within normal limits in most cases. This mechanism has been found in normal subjects (see Chapter III). In fact, the intracellular potassium concentration was slightly above the normal range in 5 cases. The interpretation of this finding is difficult; it is certainly not what would be expected if the cell membrane was functioning poorly, and it may be that the extracellular water content has been over-estimated in these cases. The serum potassium concentration and the total potassium content were widely variable

in these 5 patients, and do not relate to the intracellular potassium content in any way differently from the other patients.

On the other hand the intracellular sodium concentration was raised in 3 patients. This may be due to inefficient working of the cell sodium pump, or to an increase in cell membrane permeability, but in each case the biopsy-specimen potassium content and the intracellular potassium concentration were quite normal, and it can be assumed from this that intracellular homeostasis has not been grossly upset.

Despite doubt over the basic mechanisms, it is generally held that hypertension, at least in the more severe degrees of chronic renal failure, is intimately related to retention of water and salt[15, 16]. The finding in this series of a good correlation between diastolic blood-pressure and extracellular water content of the biopsy-specimens gives support to this view. The fact that this relationship is more direct in the later stages of renal failure raises the possibility that factors other than simple water retention are concerned in the aetiology of hypertension in mild to moderate renal failure[17]. Although the failure to demonstrate a relationship between diastolic blood-pressure and total biopsy-specimen sodium content at first seemed surprising, there is other evidence to suggest that hypertension in severe degrees of chronic renal failure may relate more to water retention than to

sodium retention. Merril, et. al.[18] noted that hypertension developed in renoprival patients when they became over-hydrated, and that these patients often developed hyponatraemia during the hypertensive episodes. Shaldon[15] found that although inter-dialytic weight gain (i.e. fluid retention) in patients on regular dialysis was closely related to hypertension, increased salt intake was not necessary for the rise in blood-pressure to occur. He also showed that hyponatraemia often occurred when excessive inter-dialytic fluid intake had produced a rise in blood-pressure. Vorburger, et. al.[19], using multiple isotope-dilution techniques demonstrated that in patients with chronic uraemia, hydration correlated well with changes in blood-pressure. The evidence obtained from muscle biopsy-specimen analysis in this paper suggests that in the later stages of chronic renal failure extracellular water retention predominates over salt retention, with the production of a dilutional hyponatraemia, and also that hyponatraemia is more common in the hypertensive patients.

However, whether water or salt retention is the more important factor in the development of hypertension in chronic renal failure, or whether both are intimately related, there is certainly no doubt that efficient removal of the retained water and salt by regular dialysis[15], or by renal transplantation[20], will control the hypertension in the majority of these patients.

SUMMARY

Water and electrolyte status in chronic renal failure has been measured by analysis of skeletal muscle biopsy-specimens, and the results found have been compared to those of a series of normal subjects.

Extracellular water, sodium and chloride contents tended to be raised in chronic renal failure, and the changes tended to be more marked as the severity of renal impairment increased.

Intracellular water and potassium contents were normal in all but 5 of the patients. Intracellular potassium concentrations were significantly raised in 5 patients and reduced in only one. Intracellular sodium concentrations were increased in 3 patients; the intracellular potassium concentration was normal in these cases. There were significant correlations between extracellular water and sodium contents, and between intracellular water and potassium contents, which were similar to the correlations previously shown in normal subjects. These results suggest that there is surprisingly little upset of cellular function in chronic renal failure.

There was a significant correlation between diastolic blood-pressure and extracellular water content, which was especially marked in more severe degrees of renal failure. These results support the belief that hypertension in chronic renal failure

is salt and water dependent, and that hypertension in these patients can be controlled by removal of salt and water.

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CHAPTER VIII

BODY WATER AND ELECTROLYTE COMPOSITION IN ACUTE RENAL FAILURE

INTRODUCTION

Acute renal failure is a not uncommon illness in hospital practice. Numerous aetiological factors have been documented[1-4], but it has been stated that at least 30% of cases cannot be attributed to a single causal agent[1, 5]. It is well recognised that conservative fluid and electrolyte therapy, as first advocated by Strauss[6] and Bull, et. al.[7], allied with dialysis treatment at an early stage[8, 9], can keep patients with acute renal failure in virtually normal water and electrolyte balance throughout the oliguric phase. However, although certain clinical situations are known to predispose to acute renal failure and its onset may therefore be noted quickly, it often develops as a result of several apparently minor factors, and considerable upset of fluid and electrolyte balance may occur before the diagnosis is made.

There have been surprisingly few attempts to measure body water and electrolytes in untreated acute renal failure. Patients have been reported as showing two main types of imbalance; one where salt and water are present in excess giving overhydration, oedema and acute cardiac failure[5, 6, 10-12], and the other where body water and electrolytes are normal despite severe extracellular acidosis, uraemia and often hyperkalaemia[5, 12]. Different types of acute renal lesion may <u>per se</u> produce different patterns of

fluid and electrolyte imbalance, but probably the most important determining factor is the fluid and electrolyte intake of the patient prior to the onset of acute renal failure, and immediately thereafter.

This chapter presents the results of muscle biopsy analysis in a series of patients presenting to the renal physicians, once the diagnosis of acute renal failure had been made, but before specific therapeutic measures had been started.

PATIENTS AND METHODS

The criteria for the diagnosis of acute renal failure are the same as those laid down by Brown, et. al.[13] namely, diminution of urine volume to 500 ml. or less in 24 hours, blood urea concentration over 130 mg. per 100 ml., and a urine/blood urea ratio of 10:1 or less.

Twelve patients with acute renal failure have been studied. Eleven were studied during the oliguric phase of their renal failure, and none had received dialytic treatment. One further patient was studied during the diuretic recovery phase.

Biopsy-specimens were taken from the vastus lateralis muscle in each case, and the results have been compared to the mean values of the group of 42 normal subjects. Biopsy-specimens were

TABLE XXV

AGE, AETIOLOGICAL FACTORS AND CLINICAL CONDITION AT TIME OF BIOPSY, SERUM-ELECTROLYTE VALUES AND URINE UREA CONCENTRATION OF THE PATIENTS WITH ACUTE RENAL FAILURE

PATIENT	AGE	AETIOLOGICAL FACTORS AND CLINICAL CONDITION	SERUM-ELECTROLYTE CONCENTRATIONS (meq. per litre)				(mg. per 100 ml.)	URINE UREA (g. per litre)
			SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA	
1	67	Choledochotomy: hypotension: biliary fistula	115	6.7	88	8	238	7.2
2	66	Uretero-colic anastomosis: vomiting: dehydrated	141	3.4	102	16	260	3.2
3	53	Hysterectomy: paralytic ileus: hypotension: dehydrated	127	5.1	88	6	384	7.0
4	34	CCl_4 ingestion: vomiting and diarrhoea: controlled fluid intake	134	4.5	88	20	333	2.1
5	32	Acute glomerulonephritis: vomiting: poor fluid intake	133	5.8	90	11.5	285	2.6
6	57	Haematemesis: hypotension: vagotomy and gastroenterostomy: gastric suction: dehydrated	144	5.8	95	19.5	224	5.2
7	62	Endarterectomy: septicaemia: hypotension: controlled fluid intake	132	4.0	87	15	278	4.2
8	68	Choledochotomy: septicaemia: oedematous	132	5.2	93	20	186	4.8
9	53	Whipple's operation: pancreatic fistula: oedematous	118	3.4	90	15	228	4.8
10	44	Bilateral ureteric obstruction: carcinoma cervix: oedematous	138	4.5	91	26.5	184	-
11	71	Prostatic hypertrophy: catheterisation: septicaemia: hypotension: oedematous	133	5.1	92	15	248	1.8

TABLE XXVI

INDIVIDUAL BIOPSY-SPECIMEN VALUES FOR PATIENTS WITH ACUTE RENAL FAILURE

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
1	3403	577	2826	94	465	61	10	163	3.7
2	2856	672	2184	94	377	78	4	172	4.3
3	2971	645	2325	101	379	65	8	162	3.7
4	3169	1010	2159	109	420	98	10	192	3.7
5	3372	725	2647	111	453	75	7	170	3.8
6	2989	409	2580	119	449	48	24	173	3.9
7	3151	858	2293	140	398	82	12	173	3.3
8	4195	1337	2859	170	464	136	3	159	3.9
9	3975	1535	2440	207	378	147	11	153	3.7
10	4226	1337	2889	274	445	133	31	152	3.8
11	4255	1490	2765	280	481	153	30	172	3.8

TABLE XXVII

MEAN BIOPSY-SPECIMEN RESULTS OF THE TWO GROUPS OF PATIENTS WITH ACUTE RENAL FAILURE, COMPARED TO THE NORMAL MEAN VALUES

GROUP		BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
		TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMALS (n=42)	MEAN	3187	703	2483	136	429	82	15	172	4.3
	S.D.	168	135	180	27	32	14	7	8	0.2
PATIENTS 1 - 7	MEAN	3130	699	2431	110	420	72	11	172	3.8
	S.D.	206	194	255	16	36	16	6	10	0.3
PATIENTS 8 - 11	MEAN	4163	1439	2740	233	442	142	19	159	3.8
	S.D.	128	118	206	53	45	9	13	9	0.1

TABLE XXVIII

STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN THE NORMAL VALUES AND THE TWO GROUPS OF ACUTE RENAL FAILURE

GROUP	BIOPSY-SPECIMEN WATER			TOTAL BIOPSY-SPECIMEN ELECTROLYTES			INTRACELLULAR ELECTROLYTES		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMAL vs. 1 - 7	N.S.	N.S.	N.S.	$P<0.02$	N.S.	N.S.	N.S.	N.S.	$P<0.001$
NORMAL vs. 8 - 11	$P<0.001$	$P<0.001$	$P<0.01$	$P<0.001$	N.S	$P<0.001$	N.S.	$P<0.005$	$P<0.001$

N.S. = not significant

taken, analysed and partitioned as previously described.

RESULTS

Clinical details and serum-electrolyte concentrations of the 11 patients with acute renal failure are shown in Table XXV. Normal serum-electrolyte ranges are:- sodium, 135 - 148 meq. per litre; potassium, 4.1 - 5.5 meq. per litre; chloride, 96 - 106 meq. per litre; and bicarbonate, 24 - 31 meq. per litre. Hyponatraemia and hypochloraemia are common, being seen in 8 and 10 patients respectively. Hyperkalaemia is present in 3 cases, and hypokalaemia in 2 cases. All had low serum bicarbonate concentrations.

Individual biopsy-specimen results of these patients are shown in Table XXVI, and it is obvious that they fall into two groups, patients 1 - 7 having virtually normal water values, and normal or low sodium and chloride contents, and patients 8 - 11 showing overhydration and sodium and chloride excess. The mean biopsy-specimen values of these two sub-groups are compared to the normal mean values in Table XXVII, and the statistical significance of the differences is shown in Table XXVIII. The results may be summarised thus:-

(1) Patients 1 - 7: total, extracellular and intracellular

TABLE XXIX

BIOPSY-SPECIMEN RESULTS IN A PATIENT IN THE DIURETIC PHASE OF ACUTE RENAL FAILURE

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
12	3162	592	2569	77	463	61	5	179	3.5

water contents do not differ from normal; the total biopsy-specimen sodium content is significantly lower than normal, and thus the hyponatraemia seen in most of these patients is the result of a sodium deficit. The intracellular chloride concentration is significantly reduced, but all the other values are normal.

(2) Patients 8 - 11: Total and extracellular water and sodium and chloride contents are significantly increased; the hyponatraemia in these cases is therefore dilutional, extracellular water being increased to a greater degree than the sodium content. Intracellular water is also significantly increased, but the mean biopsy-specimen potassium content does not differ significantly from normal; as a result of this the mean intracellular potassium concentration is significantly reduced. The intracellular sodium concentration is above the normal range in two individual patients, but the mean value for the group does not differ from normal.

One patient was studied during the diuretic recovery phase of acute renal failure. Urine volumes were normal for 4 days before muscle biopsy was performed, and the patient was taking an 18 G. Giovanetti diet, fluid ad libitum and potassium supplements. Serum-electrolyte concentrations were:- sodium, 134 meq. per litre; potassium, 3.9 meq. per litre; chloride, 83 meq. per litre; bicarbonate, 34 meq. per litre; and urea 240 mg. per 100 ml. The biopsy-specimen results are shown in Table XXIX. Total sodium

and chloride contents are both low; all other values are within the normal ranges. Urine sodium concentration at this time was high, being 60 - 70 meq. per litre. Additional sodium intake was not given, and 3 days later the serum sodium concentration was 124 meq. per litre. Urine sodium concentration was however, only 4 meq. per litre, and the serum sodium concentration rose quickly to normal thereafter.

DISCUSSION

The evidence given in the previous chapter on the effect of uraemia on the cell and cell membrane was derived mainly from acute experimental uraemic conditions. It seems likely therefore that patients with acute renal failure will have cell membrane potentials little altered from normal, and that the methods used in this thesis to estimate the extracellular space of the biopsy-specimens will be satisfactory in these patients. Furthermore, Merrill[1] has stated on the basis of his extensive studies, that entry of chloride into the cell does not appear to be a complication in acute renal failure.

The first sub-group of patients with acute renal failure shows a marked tendency towards a reduction in sodium content, and to a lesser extent chloride content, all other values being very

close to normal. There are two possible explanations for this. Firstly, a defect of tubular reabsorption is often the first stage of the pathological lesion of acute renal failure, and such patients may lose large quantities of electrolyte in the urine[3, 15-17]. Secondly, all these patients had conditions likely to lead to extrarenal loss of water and electrolyte, such as vomiting, gastric suction, paralytic ileus and diarrhoea; the clinical and biopsy-specimen evidence suggests that replacement of these extrarenal losses had been inadequate in many of these patients.

The second sub-group of patients with acute renal failure shows evidence of marked generalised overhydration, associated with an excess of sodium and chloride. The intracellular potassium concentration is significantly reduced, due to the marked increase of intracellular water with only slightly increased potassium content. In retrospect, this situation arose either from an initial failure to recognise the development of acute renal failure, or from the mistaken belief that the oliguria in these patients was the result of dehydration; excessive administration of water and electrolyte was the result.

Therefore, although the treatment of the oliguric phase of acute renal failure has been well described[1, 3, 4, 18], and has been largely responsible for the marked fall in mortality, these biopsy-specimen results show that the body water and electrolyte

content of patients presenting for the treatment of acute renal failure can be very variable. It is difficult, especially where extra-renal losses have occurred, to assess fluid and electrolyte imbalance on the basis of clinical judgement, and serum-electrolyte values may be difficult to interpret. For instance hyponatraemia is an extremely common finding in acute renal failure, and was noted in this series in patients with low and with high biopsy-specimen sodium contents. These patients all had sources of extra-renal fluid and electrolyte loss, but the first group had been given inadequate, and the second excessive replacement of these losses, whether by accident or by design. The view widely quoted that hyponatraemia in acute renal failure is a dilutional phenomenon[1, 3, 5, 10, 18, 19], may be true for most patients with a "medical" or "obstetrical" cause for their renal failure, but should obviously be treated with suspicion in post-surgical cases, who nearly all have extrarenal fluid and electrolyte loss. It would seem wise therefore to measure body water and electrolyte contents in such patients, either by isotope-dilution or by muscle biopsy, prior to the start of treatment.

Many authors have reported that electrolyte loss in the urine may be marked in the diuretic recovery phase of acute renal failure[3, 10, 12, 15, 19]. It has been suggested that the excess electrolyte excreted in the urine is that which has accumulated

in excess of normal during the oliguric phase[3, 16, 17]. There is good evidence to suggest that the patient studied in this series had been treated accurately during the oliguric phase of his illness and was not overloaded with fluid or salt. However, after only 4 days of diuresis he showed biopsy-specimen evidence of sodium and chloride depletion, and had therefore lost more than purely excess electrolyte. The final outcome in this patient was complete recovery, but obviously such an electrolyte depletion could have been dangerous. It seems likely therefore that severe electrolyte losses can occur during the diuretic phase, and adequate replacement therapy should be given during this period.

SUMMARY

Biopsy-specimen water and electrolytes have been measured in 11 patients with untreated acute renal failure, and in one patient during the diuretic recovery phase of his illness.

Patients with acute oliguric renal failure fall into two main groups. The first group shows evidence of a reduction in extracellular sodium and chloride, with normal intracellular water and electrolytes. These findings are thought to be due to a combination of excess urinary salt loss during the development of oliguric renal failure, and inadequate replacement of extrarenal electrolyte losses.

The second group shows overhydration of both extracellular and intracellular phases, associated with an excess of sodium and chloride. Due to intracellular water excess the intracellular potassium concentration is reduced.

The patient studied during the diuretic recovery phase of acute renal failure showed a marked loss of sodium and chloride, and shows the necessity to replace urinary electrolyte losses at this stage of the illness.

It is often extremely difficult to assess fluid and electrolyte balance in patients presenting for the treatment of acute renal failure, and muscle biopsy analysis or isotope-dilution studies may be required before accurate replacement therapy is possible.

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CHAPTER IX

MUSCLE BIOPSY ANALYSIS IN CONGESTIVE CARDIAC FAILURE

INTRODUCTION

The problem of body water and electrolyte imbalance in congestive cardiac failure has received considerable attention for many years; however, the number of published reports only bear witness to the conflicting results found in many instances.

There is no doubt that patients with severe congestive cardiac failure, who are oedematous, have an expansion of total and extracellular water, and sodium and chloride contents, measured by muscle biopsy analysis[1-9], or by isotope-dilution studies[10-15]. In non-oedematous patients however, it has been reported both that the body sodium content is still greater than normal[10, 11, 13, 14] and that it is within normal limits[12, 19, 20].

Body exchangeable potassium contents have been found to be lowered in congestive cardiac failure[11, 12, 16, 19-22]. However, wasting is common in patients with severe heart disease, though the presence of oedema may mask the weight loss. Under these circumstances a reduction in potassium content, as measured by isotope-dilution, may be an indication of loss of lean body mass, with little alteration in intracellular potassium content. The results of muscle biopsy analysis, which might have been expected to clear confusion on this matter, have been variable; biopsy-specimen potassium content has been reported as normal[5, 6, 9, 23] or low[1-3, 7, 8], and intracellular potassium concentration has also

TABLE XXX

AGE, TREATMENT AND SERUM ELECTROLYTE CONCENTRATIONS OF ALL PATIENTS WITH CONGESTIVE HEART FAILURE

PATIENT	AGE (YEARS)	TREATMENT	SERUM ELECTROLYTE CONCENTRATIONS (meq. per litre)				(mg. per 100 ml.)
			SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA
SEVERE C.C.F.							
1	62	DIURETIC + DIG. + K	131	3.1	93	24	84
2	51	DIURETIC + DIG.	126	6.1	86	10	136
3	65	DIURETIC + DIG. + K	141	3.3	102	27.5	64
4	62	DIURETIC + DIG.	136	4.5	99	21.5	97
5	42	DIURETIC + DIG. + K	134	4.5	91	34.5	28
6	42	DIURETIC + DIG.	129	4.2	78	35	94
CONTROLLED C.C.F.							
7	53	DIURETIC + DIG. + K	140	4.7	99	31.5	17
8	37	DIURETIC + DIG. + K	133	4.2	93	28	38
9	41	DIURETIC + DIG. + K	141	4.5	105	23	51
10	42	DIURETIC + DIG.	141	3.7	106	25	64
11	63	DIURETIC + K	140	4.3	101	31.5	32
12	63	DIURETIC + K	143	3.8	102	28	41
13	60	DIURETIC + K	141	3.9	99	28.5	36
14	46	DIURETIC + K	141	3.5	99	30	45

DIG. = digoxin or related compounds
K = potassium supplements

been found to be normal or low.

Finally some authors believe that cells gain sodium and/or chloride[5, 9, 24], at least in severe congestive cardiac failure; but this has not been noted in other published reports[11, 12, 15].

This present study has been carried out to examine possible causes for these variable findings reported above.

PATIENTS AND METHODS

Fourteen patients with congestive cardiac failure have been studied, 6 with severe uncontrolled oedematous cardiac failure, and 8 who had been oedematous but had responded well to treatment and were non-oedematous at the time of biopsy. All patients were receiving treatment as shown in Table XXX.

All biopsy-specimens were taken from vastus lateralis muscle, and the results have been compared to the mean values of the group of 42 normal subjects previously described.

Biopsy-specimens were taken, analysed and partitioned as described in Chapter II.

RESULTS

Table XXX shows the age, treatment and serum-electrolyte values of the two groups of patient. Normal serum electrolyte

TABLE XXXI

INDIVIDUAL BIOPSY-SPECIMEN RESULTS OF ALL PATIENTS WITH CONGESTIVE CARDIAC FAILURE

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
SEVERE C.C.F.									
1	3771	1238	2487	271	375	129	41	149	3.9
2	3887	1349	2528	200	427	135	13	166	3.6
3	3707	1252	2455	196	369	138	8	149	4.3
4	4761	2517	2243	355	363	258	15	157	4.1
5	3987	1715	2272	254	413	165	11	178	3.8
6	4404	1647	2738	291	475	138	28	171	3.3
CONTROLLED C.C.F.									
7	2966	592	2374	110	417	68	11	174	4.1
8	3139	697	2442	130	385	73	15	161	3.8
9	3143	1158	1985	209	341	130	23	169	4.4
10	3449	1054	2412	192	430	123	18	177	4.4
11	3340	892	2449	153	406	100	12	164	4.2
12	3274	1102	2171	205	407	122	21	187	4.3
13	3332	1162	2170	160	422	124	2	192	4.1
14	3082	996	2087	144	373	107	2	177	4.1

TABLE XXXII

MEAN BIOPSY-SPECIMEN VALUES FOR TWO GROUPS OF PATIENTS WITH CONGESTIVE CARDIAC FAILURE COMPARED TO THE MEAN NORMAL VALUES

GROUP	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMAL									
MEAN	3187	703	2483	136	429	82	15	172	4.3
S.D.	168	135	180	27	32	14	7	8	0.2
SEVERE C.C.F.									
MEAN	4086	1627	2454	261	404	161	19	162	3.8
S.D.	412	477	182	60	43	49	13	12	0.3
CONTROLLED C.C.F.									
MEAN	3216	957	2261	163	398	106	13	175	4.2
S.D.	160	214	180	36	29	24	8	11	0.2

TABLE XXXIII

STATISTICAL SIGNIFICANCE OF ANY DIFFERENCE BETWEEN GROUPS SHOWN IN TABLE XXXII

GROUP	BIOPSY-SPECIMEN WATER			TOTAL BIOPSY-SPECIMEN ELECTROLYTES			INTRACELLULAR ELECTROLYTES		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMAL vs. SEVERE C.C.F.	P<0.001	P<0.001	N.S.	P<0.001	N.S.	P<0.001'	N.S.	P<0.01	P<0.001
NORMAL vs. CONTROLLED C.C.F.	N.S.	P<0.001	P<0.005	P<0. 02	P<0.02	P<0.001	N.S.	N.S.	N.S.
SEVERE C.C.F. vs. CONTROLLED C.C.F.	P<0.001	P<0.005	N.S.	P<0.005	N.S.	P<0. 02	N.S.	N.S.	P<0. 05

ranges are:- Sodium, 135 - 148 meq. per litre, potassium, 4.1 - 5.5 meq. per litre; chloride, 96 - 106 meq. per litre, bicarbonate, 24 - 31 meq. per litre. Hyponatraemia and hypochloraemia are common in the severely ill patients, but are seen in only one case in the non-oedematous group. Hypokalaemia was present in 6 patients, and was not related to the severity of the disease.

Individual biopsy-specimen results are shown in Table XXXI. Table XXXII gives the mean biopsy-specimen values for the two groups compared to the mean normal values, and Table XXXIII shows the statistical significance of the differences between the groups. The findings may be summarised thus:

(1) Severely ill oedematous group: Each patient has significantly increased total and extracellular water, and total sodium and chloride contents. Three patients had normal intracellular water contents, but low biopsy-specimen potassium contents; they therefore had low intracellular potassium concentrations. The mean intracellular water content of this group was quite normal, but the mean potassium content was lower than normal (not statistically significant). However, as a result of this, the mean intracellular potassium concentration was significantly reduced. Only one individual patient had an intracellular sodium concentration higher than the normal range.

(2) Controlled non-oedematous group: Two patients in this group

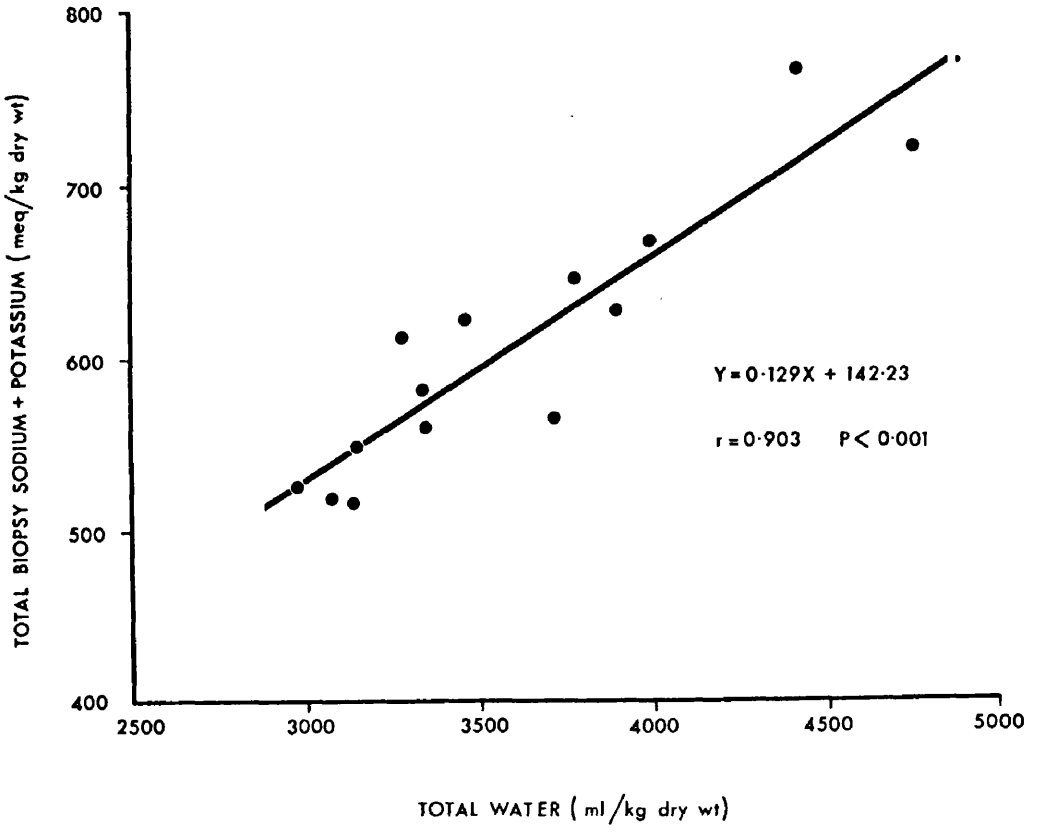

Figure 24: Correlation between biopsy-specimen total water and the sum of the total sodium and potassium contents in 14 patients with congestive cardiac failure.

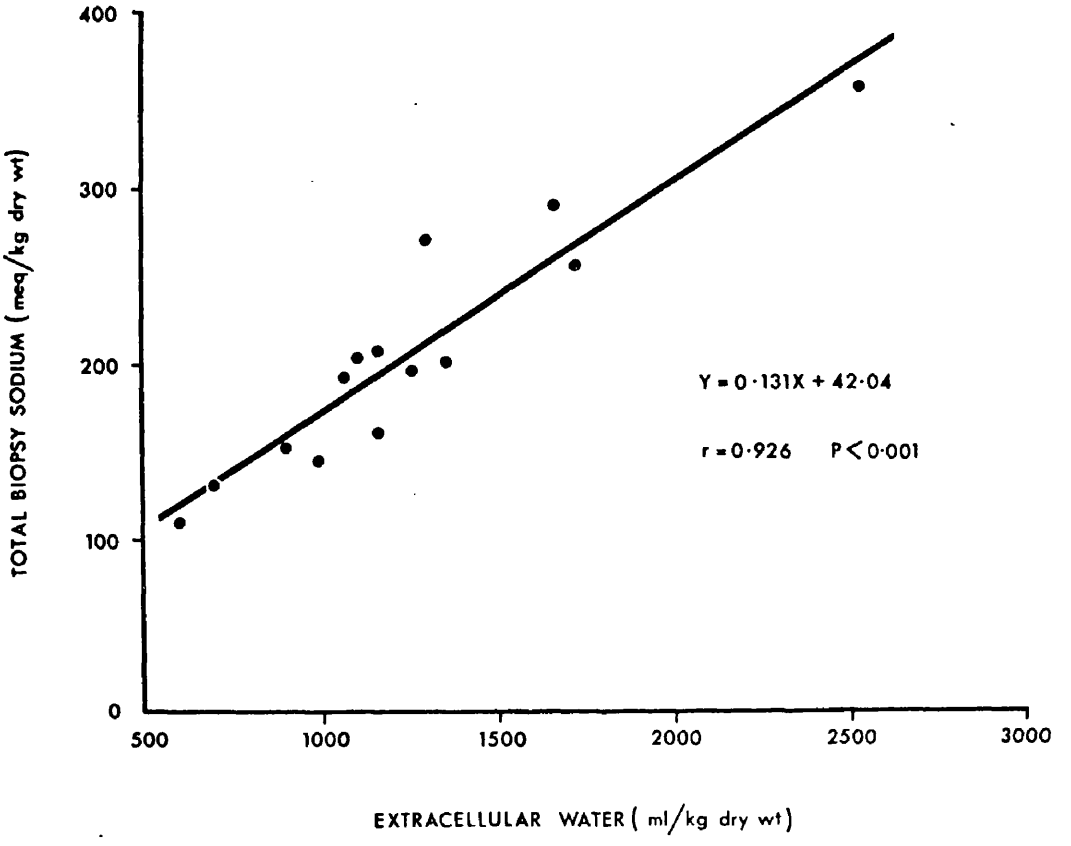

Figure 25: Correlation between biopsy-specimen extracellular water and total sodium content in 14 patients with congestive cardiac failure.

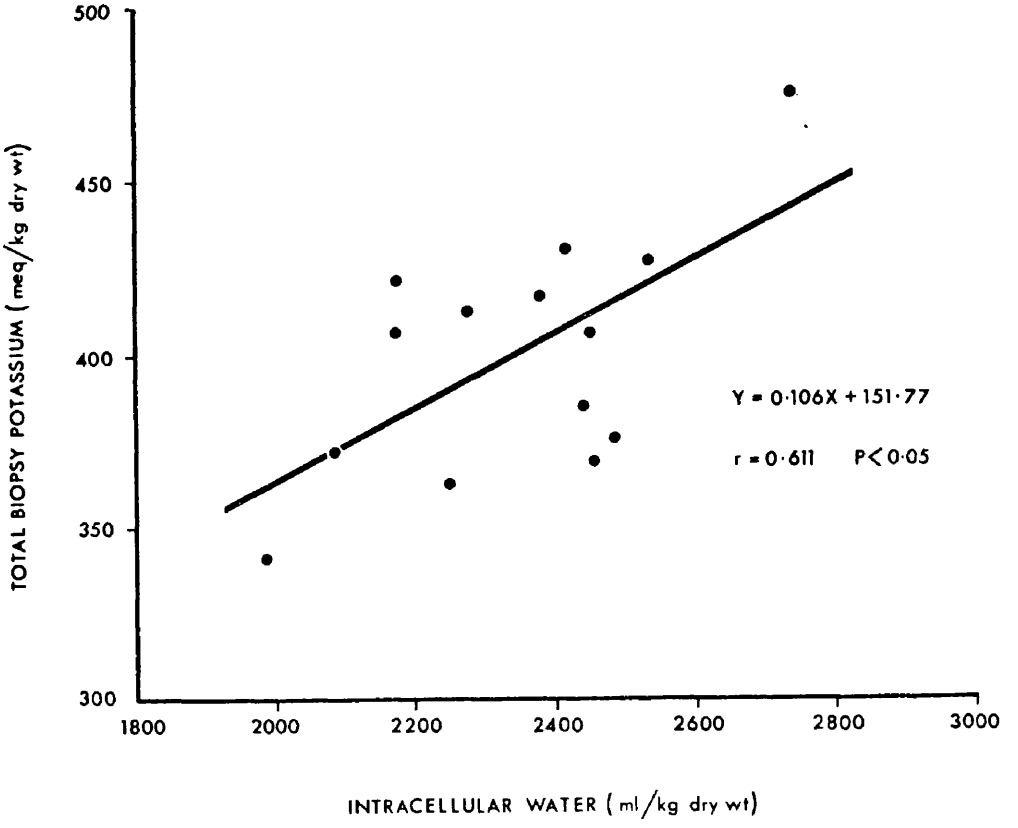

Figure 26: Correlation between biopsy-specimen intracellular water and total potassium content in 14 patients with congestive cardiac failure.

(numbers 7 and 11) have completely normal biopsy-specimen water and electrolytes. However, the mean values for extracellular water, and sodium and chloride contents are significantly greater than normal. The mean biopsy-specimen intracellular water and potassium contents are both significantly lower than normal; as both are reduced, the intracellular potassium concentration is quite normal. Intracellular sodium concentration is normal in all patients.

Mean total and extracellular water, and sodium and chloride contents are significantly lower in this group than those of the severely ill oedematous group of patients.

There is an excellent correlation between biopsy-specimen total water and the sum of the total sodium and potassium contents (Figure 24). There are also good correlations between extracellular water and sodium content (Figure 25) and intracellular water and potassium content (Figure 26).

The biopsy-specimen potassium contents do not correlate well with the severity of the clinical condition, nor with the dose or duration of diuretic therapy.

DISCUSSION

Flear, et. al.[9] have reported an increased intracellular

chloride content in patients with severe oedematous congestive cardiac failure using an *in vitro* inulin equilibration method to measure the extracellular water content of their biopsy-specimens. They did however, show a large variation in their results, and this was therefore not found in every case. They also found normal intracellular chloride concentrations in patients with mild or moderate heart failure. Mokotoff, et. al.[5] found no evidence to suggest an increased intracellular chloride concentration in the patients they studied, although these patients were probably not as ill as the ones studied by Flear and his associates. If the finding of Flear, et. al.[9] is correct, then the mean extracellular water of the severely ill patients in this study, calculated on the basis of a passive distribution of chloride across the cell membrane, will have been over estimated by 11.7% (taking the mean intracellular chloride concentration as 11.6 meq. per litre, from Flear, et. al.[9]). This will of course alter the values for intracellular electrolyte concentrations in this group, and the possible errors in these results in this study will be discussed below.

There is however, no doubt that patients with severe congestive cardiac failure show increased total and extracellular water, and sodium and chloride contents; there is total agreement in the literature on these findings. The results of the non-oedematous group of patients are more difficult to assess.

The mean biopsy-specimen results of this group suggest that extracellular water, sodium and chloride are still greater than normal, and that there is a degree of intracellular dehydration. However, these findings are not invariable, and some individual patients had quite normal or only slightly altered biopsy-specimen water and electrolytes. Therefore an obvious explanation for the findings of some authors that extracellular constituents are increased in non-oedematous cardiac failure[10, 11, 13, 14], which is denied by others[12, 19, 20], is that the groups of patients studied were dissimilar. These patients might be further sub-divided into a "totally compensated group" with normal body constituents, and a "partly compensated group" showing still greater than normal extracellular contents. Such a diagnosis however, would be difficult to make on clinical grounds, and is somewhat artificial.

Only 2 patients in this series had biopsy-specimen potassium contents significantly outwith the normal range. Four others had potassium contents lying at the lower limit of the normal range. There would appear therefore to be an actual loss of potassium from the cell in only some patients with congestive cardiac failure, being perhaps more common in severely ill oedematous patients. There is no correlation in this series between potassium depletion and diuretic therapy, although most of the patients studied were taking potassium supplements. It would again therefore seem wrong to state categorically that all patients with congestive cardiac

cardiac failure have either normal or low body potassium contents, although the mean values of both groups in this series are lower than normal, suggesting that a tendency to potassium loss does exist. The results of the intracellular electrolyte concentrations in this study are of interest. In the non-oedematous group of patients, where the biopsy-specimen potassium content is lower than normal, the intracellular water is also reduced, with the result that the intracellular potassium concentration is within normal limits in each case; there appears therefore to be normal control of the intracellular potassium concentration, as described in Chapter III of this thesis. Intracellular sodium concentrations are likewise quite normal in this series, thus confirming the findings of Flear, et. al.[9] in moderate or mild congestive cardiac failure.

In the severely ill oedematous group of patients, where the biopsy-specimen potassium content is low, the intracellular potassium concentration is also low. The first patient of this group is the only one to show any evidence of increased intracellular sodium content, associated with a reduction in potassium content; intracellular sodium concentrations are within normal limits in all other cases. Thus the combined concentration of sodium and potassium in intracellular water tends to be lower than normal, and this is a logical finding in view of the fall in extracellular

osmotic pressure, inferred from the serum-electrolyte concentrations, seen in most of these patients. If the mean intracellular electrolyte concentrations are re-calculated, using the intracellular chloride concentrations found by Flear, et. al.[9], then the mean intracellular potassium concentration falls to 150 meq. per litre, and the mean intracellular sodium concentration rises to 26 meq. per litre. This is a 27% rise in the mean sodium concentration, but if this percentage increase is added to the intracellular sodium concentrations of the individual patients, only one other patient (number 6) shows an increase in sodium concentration outwith the normal range.

The very close correlation between extracellular water (essentially calculated from the biopsy-specimen chloride content) and the sodium content in all the patients, makes it extremely unlikely that there is a significant entry of sodium into the cell in most patients with congestive cardiac failure. The only possible objection to this finding would be that if sodium and chloride enter the cell in equal amounts in severe cardiac failure, then this correlation would still hold good. Many authors utilising isotope-dilution techniques have also reported that the increase in sodium content in congestive cardiac failure is purely extracellular[11, 12, 15, 20], but their findings would also not detect an equivalent increase in intracellular sodium and chloride. However, the evidence presented here suggests that entry of sodium

into the cell probably does occur occasionally in severe oedematous congestive cardiac failure, but is an exceptional finding rather than the rule.

Finally, several authors have postulated that osmotic inactivation of intracellular cation occurs in congestive cardiac failure, in order to explain their findings of increased body cation content without a corresponding increase in body water[9, 13, 14]. However, the extremely close correlation in this series between total water and the sum of sodium and potassium contents (together comprising about 95% of the osmotically active cation of the body[25]) suggests that there is no significant osmotic inactivation of cation in this study; this agrees with the findings of Birkenfeld, et. al.[11], Olesen,[12, 15, 17, 18], and White, et. al.[20]. The consensus of opinion therefore is against osmotic inactivation, and the distribution of water and electrolytes in patients with congestive cardiac failure appears to follow the normal osmotic gradients.

SUMMARY

Biopsy-specimen water and electrolyte contents have been studied in 14 patients with congestive cardiac failure, 6 being severely ill and oedematous, and 8 well-controlled and non-oedematous at the time of biopsy.

In the severely ill patients total water, extracellular

water, and sodium and chloride contents were all grossly increased. These parameters were still greater than normal in the group of non-oedematous patients, although some patients in this group had quite normal water and electrolyte contents.

There is a general tendency to a reduction in biopsy-specimen potassium content in these patients, although only 2 individual patients had potassium contents lying outwith the normal range. In the severely ill patients, loss of biopsy-specimen potassium content was associated with a reduced intracellular potassium concentration, but in the non-oedematous patients, the intracellular potassium concentrations were normal in each case. There was no relationship between potassium loss and diuretic therapy.

Gain of sodium within the cell is an unusual finding in this series, although the method used to partition the biopsy-specimen sodium content may give an under estimate of intracellular sodium in severely ill patients.

In general, water and electrolytes are partitioned between cell and extracellular space in response to normal osmotic gradients.

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CHAPTER X

THE SYNDROME OF INAPPROPRIATE SECRETION OF ANTIDIURETIC HORMONE

INTRODUCTION

In 1938 Winkler and Crankshaw[1] published a paper entitled "Chloride depletion in conditions other than Addison's disease". They noted high urinary chloride excretion, associated with a low serum sodium concentration in two patients with pulmonary tuberculosis, and one patient with a bronchogenic carcinoma. Sims, et. al.[2] later described a syndrome of persistent hyponatraemia in patients with advanced pulmonary tuberculosis, associated with urinary hyperosmolarity and high urinary sodium concentrations. These were probably the first reports of the syndrome of inappropriate secretion of antidiuretic hormone, so named by Schwartz, et. al.[3], who showed that the symptoms of the condition could be mimicked in normal subjects by infusions of vasopressin. Epstein and Levitin[4] gave plasma from a patient with inappropriate secretion of antidiuretic hormone to a patient with diabetes insipidus, and produced a rise in urinary osmolarity and a decrease in urinary free water clearance. The cause of this syndrome was finally established beyond doubt when Bower, et. al.[5] found antidiuretic activity both in the plasma of a patient with a bronchogenic carcinoma, and also in extracts of the tumour tissue. The posterior pituitary gland of this patient had been completely destroyed by a metastatic deposit.

This syndrome has now been described in a large number

of patients, and the pathological conditions known to be associated with inappropriate antidiuretic hormone secretion have been well classified in recent review articles[6, 7].

The diagnostic features of the syndrome have been enumerated by Goldberg[6] thus:-

(1) Hyponatraemia and hypotonicity, with a urine hypertonic to plasma.

(2) Significant urinary sodium excretion despite hyponatraemia.

(3) Normal renal and adrenal function.

(4) Absence of clinical dehydration or oedema.

(5) Improvement of both hyponatraemia and urinary sodium loss by water deprivation.

This chapter reports the findings of muscle biopsy analysis of body water and electrolytes in a group of patients with inappropriate secretion of antidiuretic hormone.

PATIENTS AND METHODS

Five patients have been studied, 2 prior to any treatment, 2 before and after water deprivation, and 1 after water deprivation only. The primary pathological conditions were:- bronchogenic carcinoma, hypothyroidism, antidiuretic hormone secreting pituitary tumour, pulmonary tuberculosis (one case each), and the diagnosis

TABLE XXXIV

SERUM-ELECTROLYTE VALUES OF THE PATIENTS WITH INAPPROPRIATE SECRETION OF ANTI-DIURETIC HORMONE

PATIENT	SERUM-ELECTROLYTE CONCENTRATIONS (meq. per litre)				(mg. per 100 ml.)
	SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA
UNTREATED					
1	110	4.3	82	21.5	31
2	109	4.6	81	18.5	21
3	121	3.9	84	25.5	18
4	132	4.2	96	22.5	20
WATER DEPRIVED					
1	141	3.9	102	25.5	48
2	134	4.7	100	18.5	41
5	138	4.6	94	31	34

TABLE XXXV

INDIVIDUAL BIOPSY-SPECIMEN RESULTS OF THE PATIENTS WITH INAPPROPRIATE SECRETION OF ANTI-DIURETIC HORMONE

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
UNTREATED									
1	3723	1396	2327	169	378	122	6	163	3.4
2	3710	1280	2430	180	392	112	17	159	3.4
3	4146	1137	3009	191	447	106	16	148	3.5
4	3553	1185	2367	157	404	123	2	168	4.0
WATER DEPRIVED									
1	3307	1184	2123	179	375	130	6	175	4.3
2	3115	987	2127	172	380	108	19	176	4.2
5	3374	1003	2371	189	402	104	21	168	3.9

TABLE XXXVI

MEAN BIOPSY-SPECIMEN VALUES OF THE TWO GROUPS OF PATIENTS WITH INAPPROPRIATE SECRETION OF ANTIDIURETIC HORMONE, COMPARED TO THE NORMAL MEAN VALUES

GROUP	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMALS									
MEAN	3187	703	2483	136	429	82	15	172	4.3
S.D.	168	135	180	27	32	14	7	8	0.2
UNTREATED									
MEAN	3783	1250	2533	174	405	116	10	159	3.6
S.D.	254	114	320	15	30	8	7	9	0.3
WATER DEPRIVED									
MEAN	3265	1058	2207	180	386	114	15	173	4.1
S.D.	134	109	140	9	14	14	8	4	0.2

TABLE XXXVII

STATISTICAL SIGNIFICANCE BETWEEN GROUPS SHOWN IN TABLE XXXVI

GROUP	BIOPSY-SPECIMEN WATER			TOTAL BIOPSY-SPECIMEN ELECTROLYTES			INTRACELLULAR ELECTROLYTES		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMAL vs. UNTREATED	$P < 0.001$	$P < 0.001$	N.S.	$P < 0.01$	N.S.	$P < 0.001$	N.S.	$P < 0.005$	$P < 0.001$
NORMAL vs. H_2O DEPRIVED	N.S.	$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.001$	N.S.	N.S.	$P < 0.001$
UNTREATED vs. H_2O DEPRIVED	$P < 0.05$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

is as yet obscure in one patient. All presented with severe hyponatraemia, and on investigation all satisfied the criteria for the diagnosis of inappropriate antidiuretic hormone secretion as laid down above.

Biopsy-specimens were taken from the vastus lateralis muscle in each case, and the results have been compared to the mean values of the series of 42 normal subjects. Biopsy-specimens were taken, analysed and partitioned as described previously.

RESULTS

Serum-electrolyte values of the two groups of patients, that is untreated patients and patients after water deprivation, are shown in Table XXXIV. Normal serum-electrolyte ranges are:- sodium, 135 - 148 meq. per litre; potassium, 4.1 - 5.5 meq. per litre; chloride, 96 - 106 meq. per litre; and bicarbonate, 24 - 31 meq. per litre.

Individual biopsy-specimen results are shown in Table XXXV. The mean values of the two groups are compared to the mean normal values in Table XXXVI, and the statistical significance of any differences are shown in Table XXXVII. The results may be summarised thus:-

(1) Untreated group:- total water, extracellular water, and

total sodium and chloride contents are significantly increased. Intracellular water is greater than normal, and total potassium content is less than normal; neither reach statistical significance, but as a result of these changes the mean intracellular potassium concentration is significantly reduced. The intracellular sodium concentration is normal in all cases.

(2) Water deprived group:- comparison of patients number 1 and 2 in the two groups show the changes occurring in individual patients. Total, extracellular and intracellular water contents all fall, though the values for extracellular water still lie outwith the normal range. Total electrolyte contents are virtually unchanged, but as a result of the loss of intracellular water, the intracellular potassium concentrations have become quite normal. Water balance studies and the weight loss of these patients suggested a loss of 2,400 and 3,200 mls. of water respectively during the period of water deprivation; this is in close agreement with the water loss calculated on the basis of the biopsy-specimen results.

In the group as a whole, the mean extracellular water, and sodium and chloride contents are still significantly greater than normal; intracellular water and total potassium content are both significantly reduced. Intracellular electrolyte concentrations are quite normal.

The changes in mean water content of the two groups before and after water deprivation are quite obvious, but because of the small groups studied the reduction in total water content is the only value to reach statistical significance.

DISCUSSION

The most consistent findings of other workers studying the metabolic imbalance of inappropriate secretion of antidiuretic hormone have been an increased total body water and extracellular water[3, 7-9], and the results of this study are in agreement with these findings.

Measurement of body sodium content in such patients has given variable results. Grantham, et. al.[8] found a high sodium content in one patient studied by muscle biopsy analysis, but Kaye[10] found biopsy-specimen contents ranging from higher to lower than normal in a group of patients with inappropriate secretion of antidiuretic hormone. Total exchangeable sodium contents have been reported as normal[3, 8] or high[8]. The results of this study suggest that there is a tendency towards an increased body sodium content, although the biopsy-specimen sodium content was above the normal range in only one patient. It would seem a logical response of the body to try to increase sodium content in an attempt to restore isotonicity of the extracellular fluid, but

this finding might be somewhat unexpected, as it is well known that urinary sodium excretion is high in these patients, and they have been shown to go into negative sodium balance as hyponatraemia develops[7]. However, "steady state" conditions then prevail at a new low serum sodium concentration, and under these circumstances neutral or slightly positive sodium balances are found[6, 7, 10], even though urinary loss of sodium is still high. The sodium loss during water loading may also be replaced by mobilisation of bone sodium, thus keeping the "exchangeable" fraction of the body sodium at higher than normal levels. Therefore, the moderate increases in biopsy-specimen sodium and chloride contents may be explained in this way.

There is evidence in this study of a tendency towards potassium loss in patients with inappropriate secretion of antidiuretic hormone, which persists after fluid deprivation has restored the serum sodium concentration to normal. Balance studies have shown neutral[7] or slightly negative[11] potassium balance, but loss of body potassium has been shown in these patients, both by isotope-dilution studies[3] and by muscle biopsy analysis[8, 10]. The reduction in biopsy-specimen potassium content found in this study was never gross, but was consistent, only one patient having a potassium content greater than the normal mean value. It is not known whether the loss of potassium is a direct result of the

syndrome of inappropriate antidiuretic hormone secretion, or whether it is a manifestation of cachexia brought about by the primary pathological condition, but the findings of this study show that it is an important factor in the development of the clinical symptoms of the syndrome.

Bartter and Schwartz[7] have postulated that the symptoms occurring in patients with inappropriate secretion of antidiuretic hormone are the result of generalised hypotonicity of the body fluids, and thus of water intoxication, and the results presented here are in agreement with this concept. Intracellular hypotonicity is not so much the result of an increased intracellular water (only one patient having an intracellular water content greater than normal), but is essentially due to loss of intracellular potassium. Under normal circumstances of water metabolism, such a loss of potassium would be compensated by an equivalent loss of intracellular water, so that the intracellular potassium concentration would remain within normal limits (see Chapter III). However, because of hypotonicity of the extracellular water, and in order to maintain osmotic equilibrium across the cell membrane, water cannot leave the cell, and thus the intracellular potassium concentration falls, giving the symptoms of water intoxication. After water deprivation, water is lost from both extracellular and intracellular compartments, and the osmotic pressure on both

sides of the cell membrane returns towards normal, although now extracellular water and sodium are still present in excess and intracellular water and potassium are both lower than normal. However, as the contents of water and the principal cations are now balanced both in the extracellular phase and the cell, the serum sodium concentration and the intracellular potassium concentration both return to normal, and the patients become symptom-free.

SUMMARY

Biopsy-specimen water and electrolyte contents have been measured in 5 patients with inappropriate secretion of antidiuretic hormone, 4 prior to treatment, and 3 after water deprivation (including repeat biopsies on 2 patients).

In the untreated patients total water, extracellular water and total sodium and chloride contents were greater than normal. Intracellular water was usually normal, but total potassium content tended to be low; as a result of this the intracellular potassium concentration was significantly reduced. There was no evidence of cellular uptake of sodium.

After treatment, total water content returned to normal, but extracellular water was still higher than normal and intracellular water became significantly lower than normal.

Total electrolyte contents were unchanged, but the serum sodium and intracellular potassium concentrations were restored to normal by the loss of water.

The symptoms of inappropriate secretion of antidiuretic hormone are due to water intoxication; the hypotonicity of the extracellular fluid prevents movement of water out of the cell to balance potassium loss, and thus the intracellular potassium concentration falls. As a result of water deprivation, the intracellular water content is reduced, and the intracellular potassium concentration is restored to normal without any change in the potassium content within the cell.

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CHAPTER XI

WATER AND ELECTROLYTE METABOLISM IN DISORDERS OF ADRENAL CORTICAL FUNCTION

The patients described in this chapter all had adrenal cortical dysfunction. Three groups will be discussed, hyperaldosteronism, both primary and secondary to ischaemic renal disease, Cushing's syndrome, and adrenocorticotrophin-secreting bronchogenic carcinoma.

METHODS

Biopsy-specimens were taken from the vastus lateralis muscle in each case and the results are compared to the mean values of the group of 42 normal subjects. The biopsy-specimens were taken, analysed and partitioned as described in Chapter II.

PART 1 - HYPERALDOSTERONISM

INTRODUCTION

Primary hyperaldosteronism is an important cause of hypertension, in that surgical removal of the adrenal lesion will cure the hypertension. Conn and Louis[1] first described a hypertensive patient with hypernatraemia, hypokalaemia, and alkalosis, associated with extreme resistance to potassium repletion, and who was found to have an adenoma of one adrenal gland. Many cases have now been described in the literature, and the findings of large series have recently been published[2, 3].

With the development of techniques of assay of aldosterone and renin, it became apparent that excessive aldosterone production could also be found in hypertensive patients who did not have adrenal tumours. This "secondary hyperaldosteronism"[3] was reported in patients with ischaemic renal disease, accelerated and malignant hypertension, and often in patients with congestive cardiac failure or hepatic cirrhosis[4]. It was noted that such patients also had high plasma renin levels[5], in contrast to patients with primary hyperaldosteronism in whom the plasma renin is usually lowered[3, 6], and the high renin concentrations were thought to be the cause of the raised aldosterone levels found.

Aldosterone is known to increase renal tubular reabsorption of sodium at the expense of potassium[7], and it is of interest therefore to establish the effects of hyperaldosteronism on body water and electrolyte contents.

PATIENTS

Five patients have been studied, 3 with primary hyperaldosteronism, known to be due to adrenal adenomata, and 2 with secondary hyperaldosteronism due to unilateral renal artery stenosis. Plasma aldosterone levels were high in all the patients, ranging from 24 - 116 mμg. per 100 ml., normal values being less than 18 mμg. per 100 ml. (method of Fraser and James[8]). Plasma

TABLE XXXVIII

AGE, AETIOLOGY AND SERUM-ELECTROLYTE VALUES OF PATIENTS WITH HYPERALDOSTERONISM

PATIENT	AGE	AETIOLOGY OF HYPERALDOSTERONISM	SERUM-ELECTROLYTE CONCENTRATIONS (meq. per litre)				(mg. per 100 ml.)
			SODIUM	POTASSIUM	CHLORIDE	BICARBONATE	UREA
1	57	Adrenal adenoma: plasma renin low	142	4.0	108	27.5	32
2	57	Multiple adrenal micro-adenomata: plasma renin low	146	4.1	104	26.5	29
3	18	Adrenal adenoma: plasma renin low	150	3.0	108	27.5	18
4	38	Renal artery stenosis: plasma renin high	138	3.1	97	30.5	16
5	32	Renal artery stenosis: plasma renin high	143	3.6	107	24.5	23

TABLE XXXIX

INDIVIDUAL BIOPSY-SPECIMEN RESULTS OF PATIENTS WITH HYPERALDOSTERONISM

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
1	3221	850	2371	191	380	102	30	159	4.5
2	3162	753	2409	187	420	89	30	172	4.3
3	3172	842	2329	203	336	102	33	144	4.5
4	3419	1109	2309	216	382	117	27	164	4.0
5	3107	963	2143	195	375	113	26	174	4.5

TABLE XL

MEAN BIOPSY-SPECIMEN RESULTS OF THE GROUP OF PATIENTS WITH HYPERALDOSTERONISM COMPARED TO NORMAL MEAN VALUES

GROUP	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
NORMALS									
MEAN	3187	703	2483	136	429	82	15	172	4.3
S.D.	168	135	180	27	32	14	7	8	0.2
HYPERALDOSTERONISM									
MEAN	3216	903	2312	198	378	105	29	163	4.4
S.D.	120	137	102	11	30	11	3	12	0.2
P VALUE	N.S.	<0.005	<0.05	<0.001	<0.005	<0.001	<0.001	<0.05	<0.005

N.S. = not significant

renin levels, measured by the method of Brown, et. al.[9], were consistently low in the 3 patients with primary hyperaldosteronism, and consistently high in both patients with secondary hyperaldosteronism (normal range 4 - 20 units per litre[3]).

RESULTS

Age, aetiology and serum-electrolyte concentrations of the patients are shown in Table XXXVIII. Normal serum-electrolyte ranges are:- sodium, 135 - 148 meq. per litre; potassium, 4.1 - 5.5 meq. per litre; chloride, 96 - 106 meq. per litre; and bicarbonate, 24 - 31 meq. per litre. Only one patient had a high serum sodium concentration, but 4 of the 5 patients were hypokalaemic at the time of biopsy.

Individual biopsy-specimen results of the patients are shown in Table XXXIX, and the mean values of the group are compared to the mean normal values in Table XL. The findings may be summarised thus:- total water is normal, but there is a consistent slight expansion of extracellular water and reduction in intracellular water, the mean values being significantly greater and less than normal respectively. Total sodium and chloride contents are significantly raised, and total potassium content is significantly reduced. The mean intracellular potassium concentration is lower than normal, and the mean intracellular sodium and chloride

TABLE XLI

BIOPSY-SPECIMEN RESULTS OF A PATIENT WITH PRIMARY HYPERALDOSTERONISM

BEFORE AND AFTER TREATMENT WITH SPIRONOLACTONE

BIOPSY	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
INITIAL	3221	850	2371	191	380	102	30	159	4.5
AFTER TREATMENT (1 month later)	2942	526	2416	109	417	65	15	173	4.3

concentrations are significantly increased (the intracellular sodium concentration lying above the normal range in 3 individual patients, and at the upper limit of normal in the other 2 patients).

One patient with primary hyperaldosteronism was studied before and after treatment with the aldosterone antagonist, spironolactone. Serum-electrolyte values at the time of initial biopsy are shown in Table XXXVIII (patient number 1), and after treatment with spironolactone for one month the serum-electrolyte concentrations were:- sodium, 138 meq. per litre; potassium, 4.7 meq. per litre; chloride, 104 meq. per litre; bicarbonate, 26 meq. per litre; and urea, 30 mg. per 100 ml. Table XLI shows the biopsy-specimen water and electrolytes before and after treatment, and it can be seen that extracellular water, sodium and chloride contents have fallen, there has been a slight increase in intracellular water and potassium content, and the intracellular electrolyte concentrations have become completely normal after treatment.

DISCUSSION

Aldosterone is known to produce renal retention of sodium and to increase urinary potassium loss, and therefore these patients with hyperaldosteronism show an increase in biopsy-specimen

sodium and chloride contents and a reduction in potassium content. Similar muscle-biopsy changes have been reported in patients with primary hyperaldosteronism[1, 10], and high exchangeable sodium and low exchangeable potassium contents have been reported in 3 patients with this condition[11].

The mean intracellular potassium concentration of this group of patients is significantly lower than normal, but the individual values are variable. In a carefully controlled series of *in vitro* experiments, Adler[12] showed a significant fall in potassium content and intracellular concentration of skeletal muscle exposed to aldosterone, but he also noted variability in the response of individual muscles to aldosterone.

The intracellular sodium concentration was consistently raised in this group of patients; this has also been shown in *in vitro* studies[12, 13]. Richards, et. al.[14], however, reported that the sodium content of tissue culture cells fell after exposure to aldosterone; these were laryngeal carcinoma cells, and this fact, or the conditions of the tissue culture may explain this discrepant finding. It is interesting to note at this point that the experimental work leading to the widely held view that intracellular sodium was increased in potassium-deficiency states was carried out in animals who had been given mineralocorticoids to speed the development of potassium depletion[15-17]. Aldosterone

must produce movement of sodium into and potassium out of renal tubular cells to give its known effect on the urine, and it is an attractive hypothesis that it has a similar action on muscle cells.

This is the only group of patients studied in this thesis in which there has been any consistent evidence to suggest replacement of intracellular potassium with sodium, and it seems likely that this may be due to a direct effect of aldosterone on the cell membrane; the high intracellular sodium concentrations noted in occasional individual patients in earlier chapters of this thesis may have been a result of secondary hyperaldosteronism due to their primary illness, but no consistent effect was seen, and aldosterone secretion was not measured in these patients.

SUMMARY

Biopsy-specimen water and electrolytes have been measured in 3 patients with primary hyperaldosteronism and 2 patients with hyperaldosteronism due to ischaemic renal disease.

Extracellular water and total sodium and chloride contents were increased, and intracellular water and total potassium content were reduced in these patients.

Intracellular potassium concentration tended to be low, and there was a consistent increase in intracellular sodium

concentration, which has not been found in any other group of patients studied so far, and seems likely to be due to a direct effect of aldosterone on the cell membrane.

PART II - CUSHING'S SYNDROME

INTRODUCTION

In 1932, Cushing published the first accounts of patients with the clinical syndrome due to excessive production of cortisol by the adrenal cortex, which now bears his name[18, 19]. Since that time much has become known about the adrenal cortical malfunction in Cushing's syndrome, and about the diagnostic criteria of the condition[20], but there is surprisingly little information available regarding body water and electrolyte changes in Cushing's syndrome.

For this reason biopsy-specimen results of 2 patients with Cushing's syndrome are presented here, although any conclusions based on the findings in 2 patients only will obviously require to be treated with caution.

PATIENTS

Two patients with untreated Cushing's syndrome have been studied. Both showed the characteristic stigmata of the syndrome,

TABLE XLII

INDIVIDUAL BIOPSY-SPECIMEN RESULTS OF TWO PATIENTS WITH UNTREATED CUSHING'S SYNDROME

PATIENT	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
1	3125	1137	1988	190	341	122	15	169	4.2
2	3289	1210	2079	207	374	132	15	178	4.3

and investigation revealed high plasma cortisol values and increased urinary excretion of cortisol metabolites. The second of these patients had mild ankle oedema.

RESULTS

The serum electrolyte values of the 2 patients at the time of biopsy were (patient number 1 first):- sodium, 141, 145 meq. per litre; potassium, 3.6, 3.7 meq. per litre; chloride, 100, 102 meq. per litre; bicarbonate, 30.5, 28 meq. per litre; and urea, 36, 44 mg. per 100 ml.

Biopsy-specimen results of the patients are shown in Table XLII. Extracellular water lies above the normal range, and intracellular water below the normal range in both cases. Total sodium and chloride contents are high, and total potassium content is low. Because both intracellular water and total potassium contents are reciprocally reduced the intracellular potassium concentrations are quite normal. Intracellular sodium and chloride concentrations are also normal.

DISCUSSION

The published evidence available on water and electrolyte imbalance in Cushing's syndrome suggests that patients with this

syndrome tend to be overhydrated[21, 22], to have total exchangeable sodium contents within the normal range[20, 23] and to have total exchangeable potassium contents lower than normal[20, 22], or within the normal range[23]. The loss of lean body mass and gain in body fat commonly found in patients with Cushing's syndrome[20-23] makes interpretation of the results of isotope-dilution studies difficult, but all the above authors have noted that the ratio of exchangeable sodium to potassium is higher than normal in patients with Cushing's syndrome, suggesting gain of body sodium and/or loss of body potassium. The results of this study show that skeletal muscle has a higher sodium content and a lower potassium content per unit of muscle tissue, and this suggests an actual gain of sodium and loss of potassium from the body irrespective of changes in the content of lean tissue and fat in the body.

Biopsy-specimen extracellular water is increased in both patients, but intracellular water is reduced, and thus total water content is within normal limits. This agrees with the findings of Ernest[22], who showed that water loss during treatment of patient with Cushing's syndrome was only 700 ml. on average, and this water was thought to come from the extracellular phase.

Finally, although these patients show somewhat similar changes in biopsy-specimen water and electrolyte contents to those found in patients with hyperaldosteronism, in neither patient is

there any evidence of increased sodium entry into the cell, and the loss of potassium content from the cell is balanced by an equivalent loss of intracellular water so that the intracellular potassium concentration also remains quite normal. Adler[12] showed that cortisol did not affect intracellular potassium concentration in in vitro experiments.

SUMMARY

Biopsy-specimen water and electrolytes have been measured in two patients with untreated Cushing's syndrome.

Extracellular water and total sodium and chloride contents are greater than normal, and intracellular water and total potassium content are less than normal in both patients.

The loss of potassium is balanced by loss of intracellular water so that the intracellular potassium concentration remains normal; there is no evidence of an increase of cell sodium in these patients.

PART III - ADRENOCORTICOTROPHIN-SECRETING BRONCHOGENIC CARCINOMA

INTRODUCTION

Adrenal cortical overactivity resulting from excess production of adrenocorticotrophin from non-pituitary sources has

been recognised for some time. It is most common in association with oat-cell or anaplastic bronchogenic carcinoma, but has also been noted with other tumours, particularly of pancreas, thyroid and thymus[24, 25]. Meador, et. al.[24] were first to identify adrenocorticotrophin-like material from extracts of tumour tissue in such patients, which confirmed the aetiology of the adrenal overactivity. Although the most common endocrine abnormality found is an overproduction of cortisol, a pattern of electrolyte loss is often seen suggestive of excess mineralocorticoid activity which is extremely rare in non-malignant Cushing's syndrome[25]. These patients usually develop severe hypokalaemic alkalosis which seems to be the main differentiating factor between patients with ectopic adrenocorticotrophin secretion and non-malignant Cushing's syndrome[25, 26]. Also, patients with ectopic adrenocorticotrophin secretion usually die very quickly as a result of their primary tumour, and thus rarely develop the stigmata of Cushing's syndrome.

PATIENT AND RESULTS

The patient, a male aged 67 years, presented to his own practitioner complaining of rapid onset of ankle oedema and breathlessness. Treatment with a diuretic proved unsuccessful and he developed muscle weakness and mild mental confusion. He was then admitted to hospital for further investigation.

TABLE XLIII

BIOPSY-SPECIMEN RESULTS OF A PATIENT WITH AN ADRENOCORTICOTROPHIN SECRETING BRONCHOGENIC CARCINOMA BEFORE AND AFTER TREATMENT WITH METYRAPONE AND POTASSIUM SUPPLEMENTS

BIOPSY	BIOPSY-SPECIMEN WATER (ml. per Kg. dry wt.)			TOTAL BIOPSY-SPECIMEN ELECTROLYTES (meq. per Kg. dry wt.)			INTRACELLULAR ELECTROLYTES (meq. per litre)		
	TOTAL	EXTRACELLULAR	INTRACELLULAR	SODIUM	POTASSIUM	CHLORIDE	SODIUM	POTASSIUM	CHLORIDE
INITIAL	3516	1199	2317	248	379	115	31	163	3.7
AFTER TREATMENT (2 weeks later)	3634	869	2765	179	498	100	22	179	4.2

Chest x-ray revealed a lesion strongly suggestive of a bronchogenic carcinoma, and serum electrolytes showed a severe hypokalaemic alkalosis, full figures being:- sodium, 147 meq. per litre; potassium, 1.4 meq. per litre; chloride, 89 meq. per litre; bicarbonate, 48 meq. per litre; and urea, 39 mg. per 100 ml. A presumptive diagnosis of an adrenocorticotrophin-secreting bronchogenic carcinoma was made, and a muscle biopsy was carried out, the results of which are shown in Table XLIII (initial biopsy). Extracellular water, and total sodium and chloride contents were all increased. Total potassium content and intracellular potassium concentration were both at the lower limit of normal. The intracellular sodium concentration was raised. Further investigation at this time revealed high plasma cortisol levels and increased urinary excretion of cortisol metabolites.

Metyrapone, an 11 β-hydroxylase inhibitor, has been shown to produce clinical improvement in some cases of adrenocorticotrophin-secreting tumours[27, 28], and the patient was therefore started on this drug with the addition of parenteral potassium supplements in large amounts (100 - 160 meq. per day). Serum potassium and bicarbonate concentrations slowly returned to normal on this treatment, and the ankle oedema became less, although mental confusion remained and the patient's general condition continued to deteriorate. After 10 days, the serum-electrolyte concentrations were:-

sodium, 136 meq. per litre; potassium, 5.2 meq. per litre; chloride, 102 meq. per litre; bicarbonate, 22 meq. per litre; and urea, 48 mg. per 100 ml., and the results of a repeat muscle biopsy at this time are shown in Table XLIII (after treatment biopsy). Extracellular and intracellular water now both lay at the upper limit of the normal range, total sodium and chloride contents had fallen, and there was a marked increased in the total potassium content. Intracellular electrolytes were now quite normal.

Despite this partial biochemical improvement, the patient died 3 days after this second biopsy, before any palliative treatment of the primary lung neoplasm could be carried out. Post-mortem confirmed the diagnosis of bronchogenic carcinoma with multiple soft-tissue metastases, and also confirmed the presence of bilateral adrenal hyperplasia.

DISCUSSION

Although the development of a severe hypokalaemic alkalosis is well recognised as a complication of the syndrome due to adrenocorticotrophin-secreting tumours, there is no published data on body water and electrolyte changes in this condition.

The initial biopsy-specimen results in this patient are interesting in that despite the extremely low serum potassium

concentration the total potassium content was still within the lower limit of the normal range. This "sparing" of the cell potassium store despite severe hypokalaemia is probably a result of the extracellular alkalosis, which has been shown to increase the uptake of potassium by the cell, or at least to inhibit movement of potassium out of the cell in severe hypokalaemia (see Chapter V on pyloric stenosis). The other interesting finding is the presence of a raised intracellular sodium concentration. This was not found in either of the patients with Cushing's syndrome, but was a common finding in the group with hyperaldosteronism. Plasma aldosterone levels were not measured in this patient, but the high intracellular sodium concentration raises the possibility that hyperaldosteronism was contributing to the biochemical upset of this patient.

After treatment with metyrapone there was a fall in extracellular water, and sodium and chloride contents. There was a dramatic increase in the biopsy-specimen potassium content, the patient having apparently retained about 1200 meq. of potassium in 10 days. This would suggest that metyrapone has blocked the production of mineralocorticoid hormones to such an extent that renal retention of potassium has been almost complete, and the body potassium content has risen to an excessively high level.

The clinical course of this patient is similar to previous

reports in that despite apparent biochemical improvement with metyrapone the patient still died very quickly[27]. Patients who have had a long survival after adrenalectomy[29] or metyrapone therapy[27, 28] have been reported, but in the main the diagnosis of excess adrenocorticotrophic activity in patients with malignant tumours has been followed by rapid clinical deterioration and death. It is not clear, however, whether the development of ectopic adrenocorticotrophic activity is a terminal event in these cases or whether the biochemical upset so produced accelerates the death of these patients.

SUMMARY

Biopsy-specimen water and electrolyte contents have been measured in a patient with an adrenocorticotrophin-secreting bronchogenic carcinoma, before and after treatment with metyrapone and potassium supplements.

The initial biopsy revealed expansion of extracellular water, and sodium and chloride contents. Total potassium content and intracellular potassium concentrations were low, but to a lesser extent than would have been expected from the severity of the extracellular hypokalaemia. Intracellular sodium concentration was raised.

After treatment extracellular water, and sodium and

chloride contents were lowered. There was a marked increase in total potassium content, the value now lying above the normal range. Intracellular electrolyte concentrations were normal.

These results suggest (i) that hyperaldosteronism may contribute to the biochemical upset in this condition, and (ii) that metyrapone blocked mineralocorticoid activity in this case to such an extent that excessive retention of the administered potassium resulted.

Despite the apparent biochemical improvement after treatment the patient died within one month of developing symptoms.

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CHAPTER XII

THE RELATIONSHIPS BETWEEN EXTRACELLULAR AND INTRACELLULAR WATER AND ELECTROLYTES IN HEALTH AND DISEASE

INTRODUCTION

The aim of this chapter is to pool all the available information on biopsy-specimen water and electrolytes in health and disease, in order to examine the relationship between extracellular and intracellular content and concentration of electrolytes, and to see if the intracellular water and electrolyte of skeletal muscle may be predicted under changing conditions of extracellular water and electrolyte contents.

It is a widely held view that body cells behave as osmometers, and that water is passively distributed between cell and extracellular space to maintain osmotic equilibrium across the cell membrane. The original work on which this view was based was carried out under *in vitro* experimental conditions (see review article by Conway[1]). However, Leaf, et. al.[2] found evidence of the osmotic homogeneity of the body in *in vivo* animal experiments, and Wynn[3] showed that extracellular osmolarity in a wide variety of clinical conditions in man was a function of the total body water and electrolyte content. Edelman, et. al.[4] then published their classical paper showing the relationship between the serum sodium concentration and the total cation concentrations of the body. Nearly all abnormalities of the serum sodium concentration were explainable on this basis, and this was good evidence in favour of the concept of osmotic homogeneity of the body tissues.

TABLE XLIV

CLINICAL CONDITIONS OF 117 ILL

PATIENTS STUDIED IN CHAPTER XII

DIAGNOSIS	NUMBER OF PATIENTS
Pyloric stenosis	18
Chronic renal failure	22
Acute renal failure	12
Acute-on-chronic renal failure	8
Congestive cardiac failure	14
Adrenal cortical dysfunction	8
Uretero-colic anastomosis	6
Chronic diarrhoea	6
Diabetic comas	6
Inappropriate antidiuretic hormone secretion	5
Others	12
Total	117

This relationship will be re-examined in this chapter, and further evidence of the osmotic control of intracellular water and electrolytes will be presented.

PATIENTS AND METHODS

Biopsy-specimen water and electrolyte results from 42 normal subjects (described in Chapter III) and from 117 ill patients have been studied. Repeat biopsy-specimen results on ill patients after treatment have been included in this study, thus there are 125 biopsy-specimens from this group, making a total of 167 results in all. Many of the biopsy-specimen results have been discussed in individual chapters of this thesis, and the major clinical conditions of the ill patients are given in Table XLIV.

All biopsy-specimens were taken from the vastus lateralis muscle, and were analysed and partitioned as described in Chapter II.

RESULTS

Correlations have been studied between various serum-electrolyte concentrations and biopsy-specimen results, and the results are presented under 4 main headings.

1. Serum-electrolyte concentrations and biopsy-specimen electrolyte contents or intracellular concentrations

It has been shown before that serum-electrolyte concentrations correlate only poorly with the appropriate total body electrolyte contents[5], and these findings are confirmed in this study, the regression equations being:-

(a) serum sodium concentration (x) to total biopsy-specimen sodium content (y):-

$y = 0.775x + 55.76$ $\quad r = 0.115, \quad P > 0.05$

(b) serum potassium concentration (x) to total biopsy-specimen potassium content (y):-

$y = 1.456x + 351.76$ $\quad r = 0.138, \quad P < 0.001$

(c) serum chloride concentration (x) to total biopsy-specimen chloride content (y):-

$y = 0.992x + 2.73$ $\quad r = 0.311, \quad P < 0.001$

Similarly the serum-electrolyte concentrations correlate poorly with the appropriate intracellular electrolyte concentration (except of course for chloride, where the method of partition used assumes a constant relationship between serum chloride and intracellular chloride concentrations of 24 to 1). Regression equations for sodium and potassium are:-

(d) serum sodium concentration (x) to intracellular sodium

concentration (y):-

$y = 0.412x - 40.80$ $\quad r = 0.326, \quad P < 0.001$

(e) serum potassium concentration (x) to intracellular potassium concentration (y):-

$y = 5.423x + 146.15$ $\quad r = 0.349, \quad P < 0.001$

Although some of these regressions are statistically highly significant, it is obvious that the 95% confidence limits of the regression are such that any attempt to calculate the biopsy-specimen electrolyte content or intracellular concentration from these equations would be extremely inaccurate.

2. Biopsy-specimen water and cation contents

It has been shown by isotope-dilution studies that the sum of the total exchangeable sodium and potassium contents (comprising about 95% of the osmotically active cation of the body) correlates well with the total body water[6, 7]. This correlation shows an osmotic control of the total body water by the amount of cation within the body. Moore, et. al.[7] have also shown in normal subjects that the exchangeable sodium content (being mainly extracellular) correlates well with extracellular water, and that the exchangeable potassium content (being mainly intracellular) correlates well with the intracellular water. These correlations have been confirmed in normal subjects in this thesis (see Figures

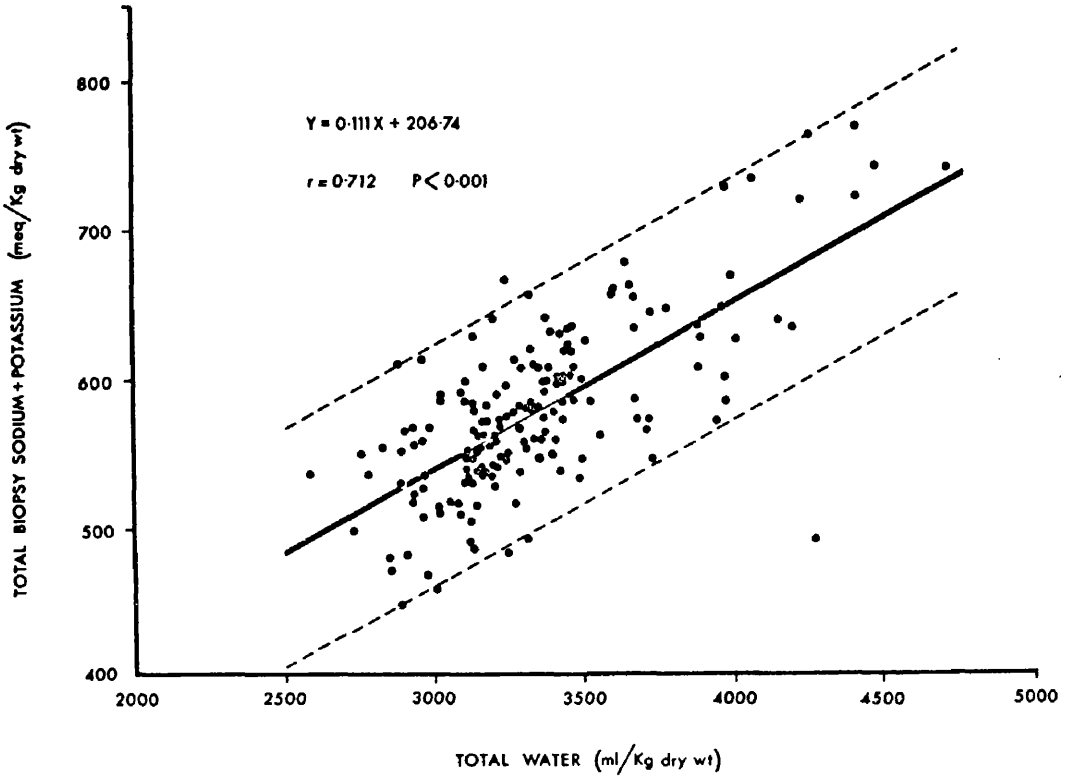

Figure 27: Correlation between biopsy-specimen total water and the sum of the total sodium and potassium contents in the whole series.

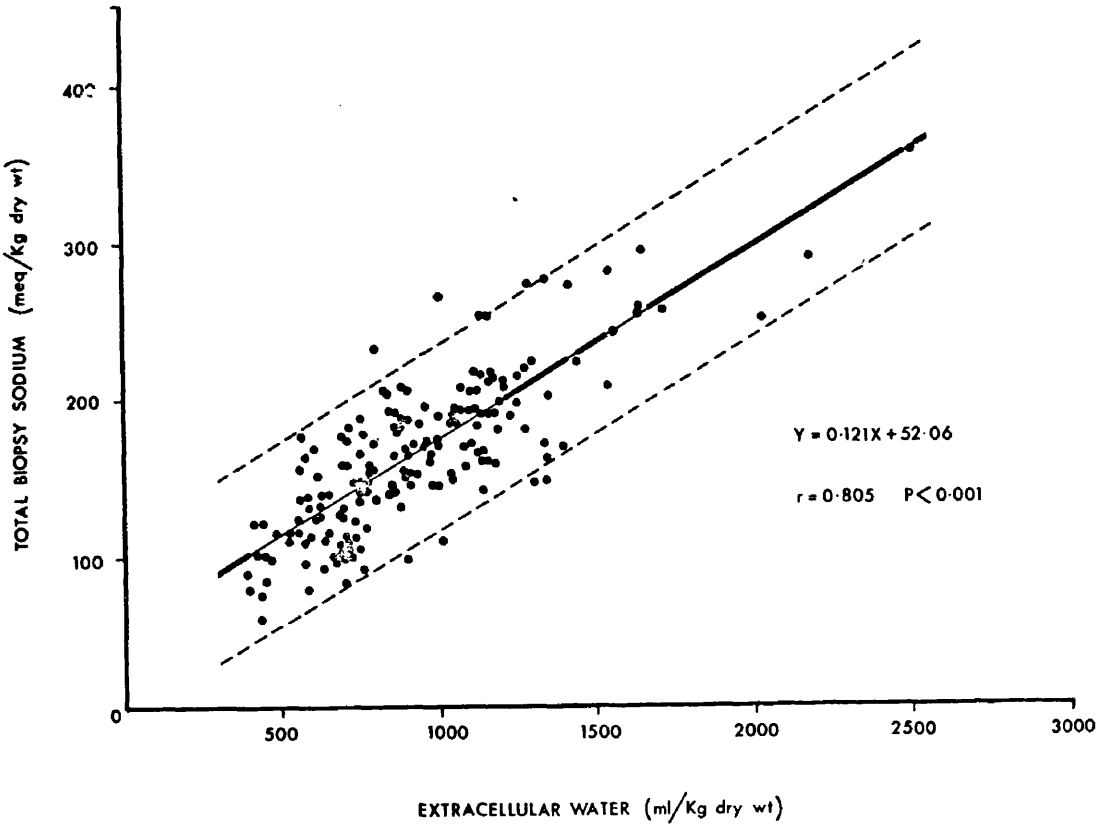

Figure 28: Correlation between biopsy-specimen extracellular water and total sodium content in the whole series.

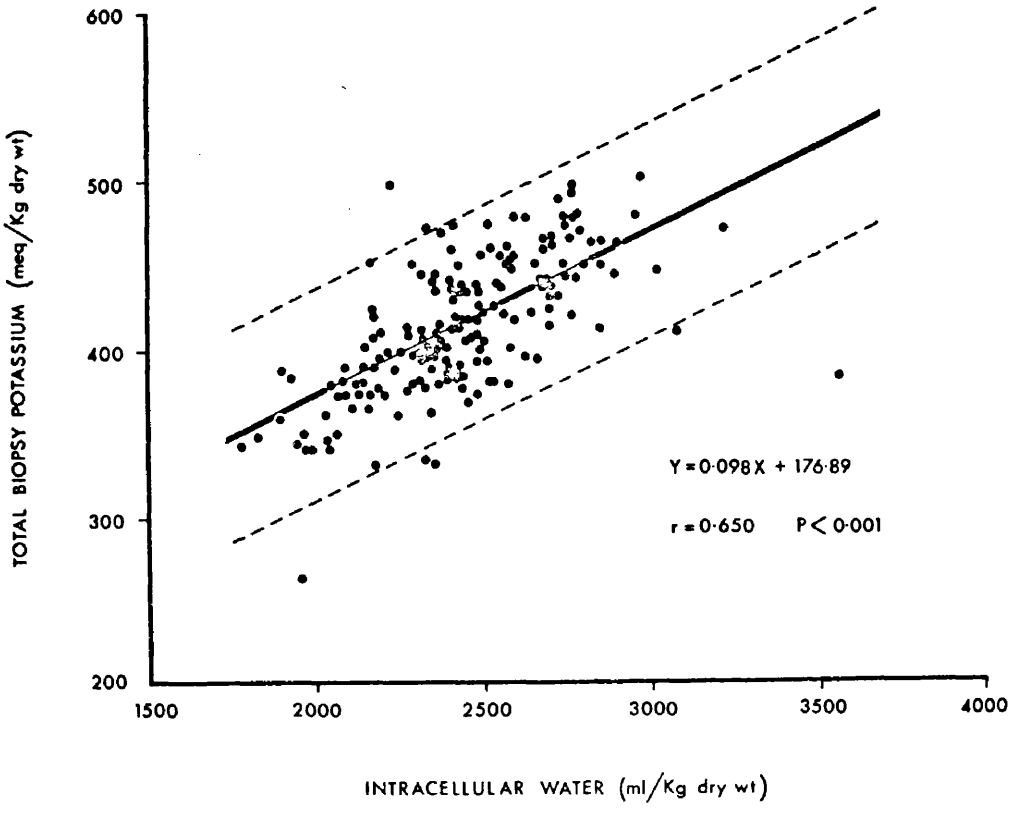

Figure 29: Correlation between biopsy-specimen intracellular water and total potassium content in the whole series.

1, 2 and 3, Chapter III).

These relationships have now been re-examined using the total material available, and the results are shown in Figures 27, 28 and 29. If the relationships between water and total electrolyte content found in normal subjects are true indications of general mechanisms of fluid and electrolyte control, then the correlations for this larger series, over a wider range of clinical conditions should be better than those for the normal series alone. This is true for the relationships between total water and the sum of the sodium and potassium contents, and for extracellular water and sodium content. However, the relationship between intracellular water and potassium content is not so close in this total series as in the normal group alone. This suggests that the concept discussed in Chapter III of reciprocal movement of water and potassium into or out of the cell, thus keeping intracellular potassium concentrations within narrow limits, only holds good under normal conditions, when it may be assumed that extracellular tonicity remains normal. The relationship of the intracellular potassium concentration to body water and electrolytes is therefore not as simple as first thought, and further relationships will be shown later, which hold good under all conditions.

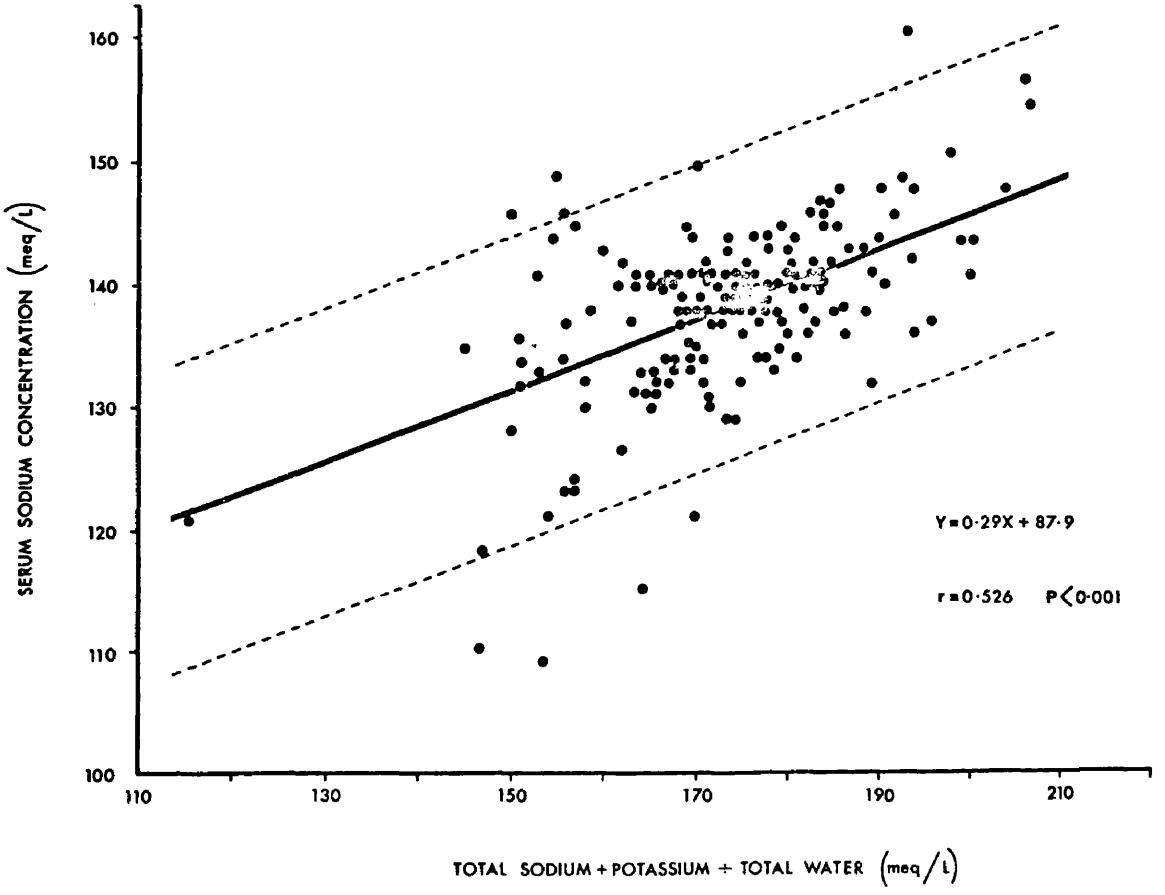

Figure 30: Correlation between total cation concentration and serum sodium concentration in the whole series.

3. Serum-electrolyte concentrations and the total cation concentration

The sum of the exchangeable sodium and potassium contents of the body, measured by isotope-dilution, comprises about 95% of the osmotically active cation of the body[7]. Therefore when this sum is divided by the total water content a figure is obtained which approximates closely to the total cation concentration of the body. Edelman, et. al.[4] were first to show that the serum sodium concentration was a function of the total cation concentration of the body over a wide range of clinical conditions. This is the best evidence to date of the passive distribution of body water across the cell membrane, and of the osmotic homogeneity between cells and extracellular fluid. Moore, et. al.[7] repeated this work, without taking into account serum water content, and found that the serum sodium concentration (y) correlated to the total cation concentration (x) in the following relationship:-

$$y = 0.333x + 84.0 \qquad r = 0.52, \quad P < 0.01$$

The same relationship in this series is shown in Figure 30, and the regression equation and correlation coefficient found are very similar to those found by Moore, et. al.[7].

This finding is really a description of the relationship between the total extracellular cation concentration (to which sodium contributes the largest fraction) and the body cation

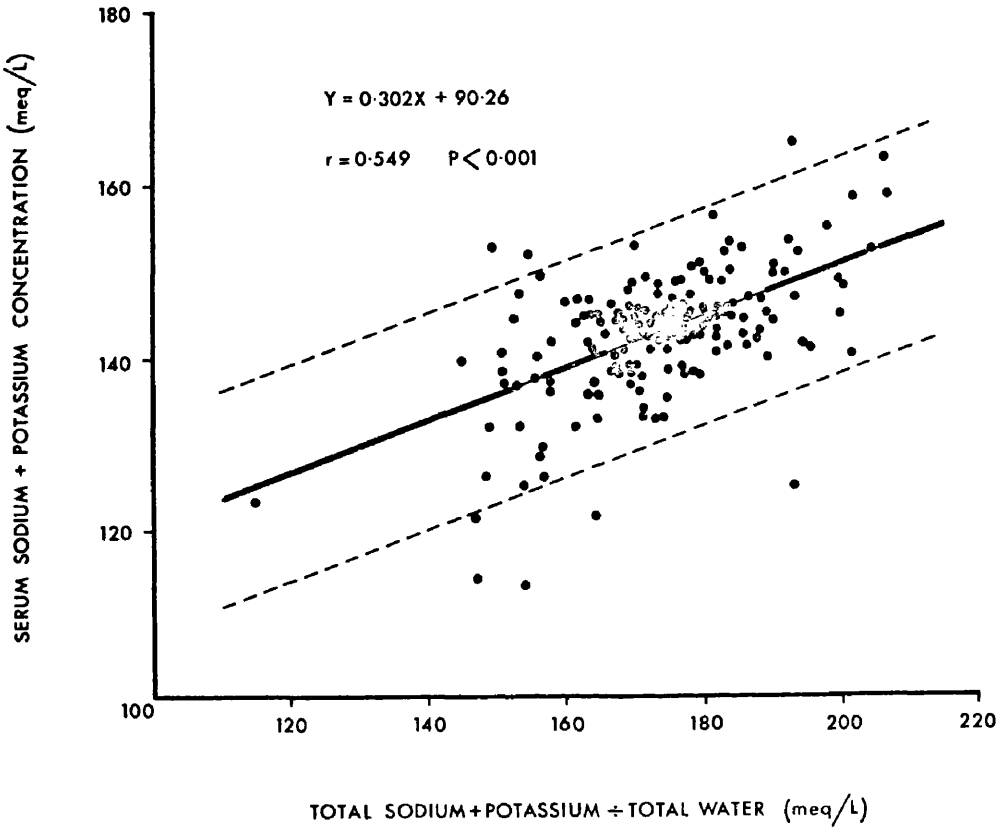

Figure 31: Correlation between total cation concentration and the sum of the serum sodium and potassium concentrations in the whole series.

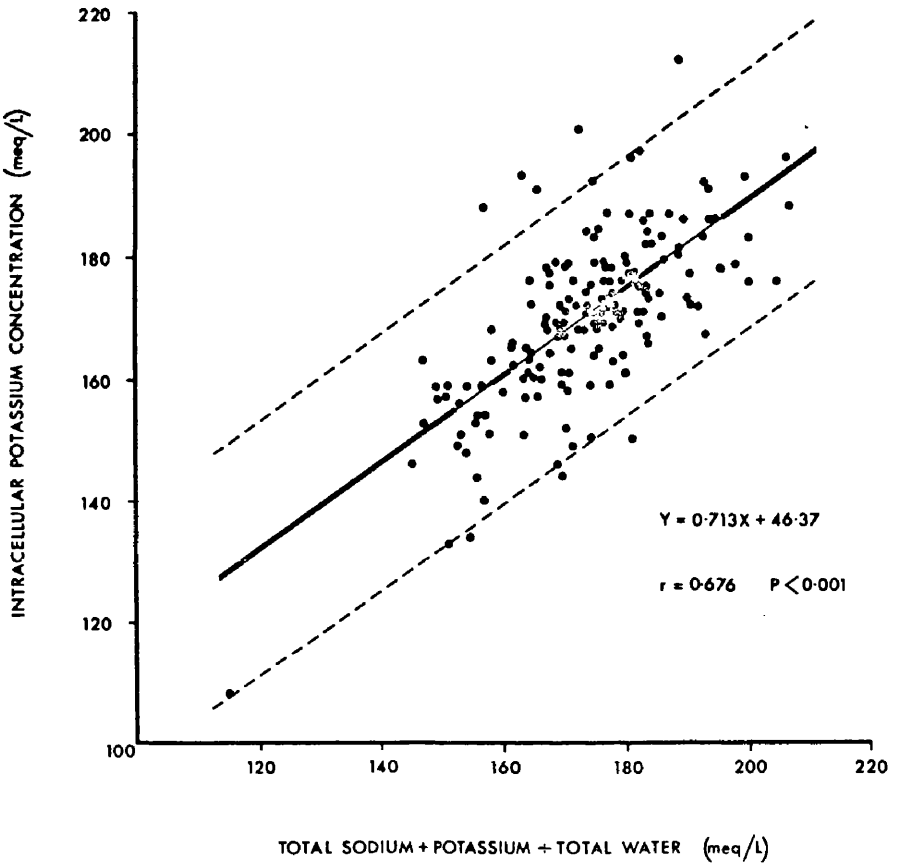

Figure 32: Correlation between total cation concentration and intracellular potassium concentration in the whole series.

concentration, and as would therefore be expected, the correlation is slightly improved when the serum sodium plus potassium concentrations are related to the total cation concentration (Figure 31).

4. Intracellular electrolyte concentrations and the total cation concentration

If the cells and the extracellular space are in osmotic equilibrium, as suggested by the correlations just described, then the intracellular potassium concentration (the principal intracellular cation) should also correlate well with the total cation concentration. This has been indeed shown in normal subjects using isotope-dilution methods[7], and also in normal subjects by biopsy-specimen analysis (see Figure 5, Chapter III).

Figure 32 shows this same relationship for the 167 biopsy-specimen results studied in this series; there is a highly significant and close correlation, and the regression equation obtained is very similar to that found in normal subjects alone.

Again, such a correlation is really a description of the relationship between the total intracellular cation concentration and the total body cation concentration, and when the sum of the intracellular sodium and potassium concentrations are related to the total cation concentration, the correlation found is extremely

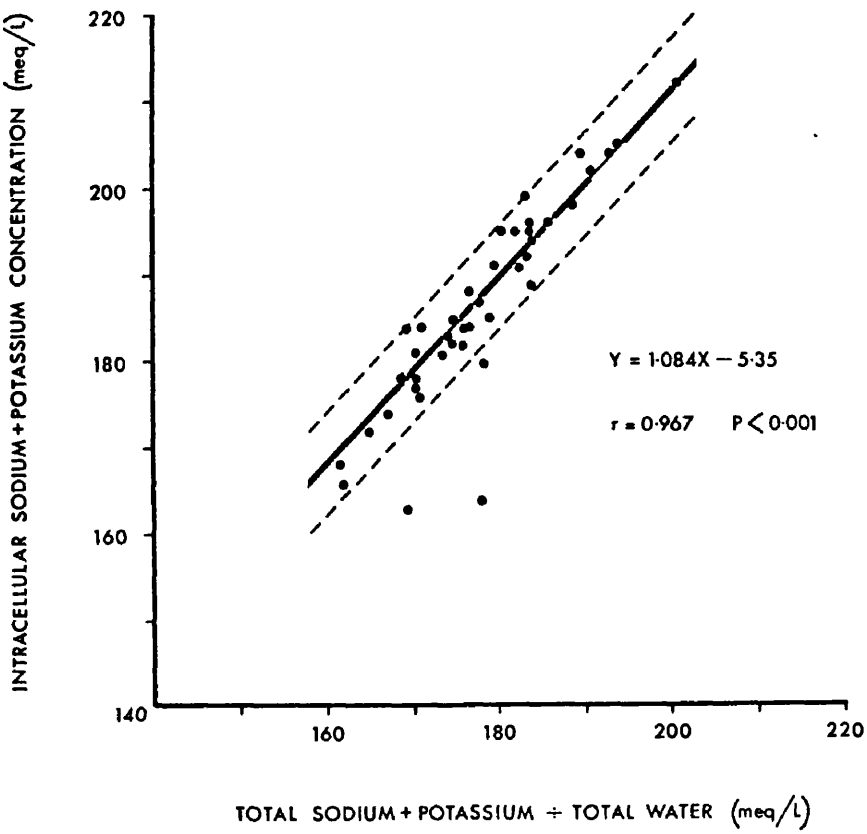

Figure 33: Correlation between total cation concentration and the sum of the intracellular sodium and potassium concentrations in 42 normal subjects.

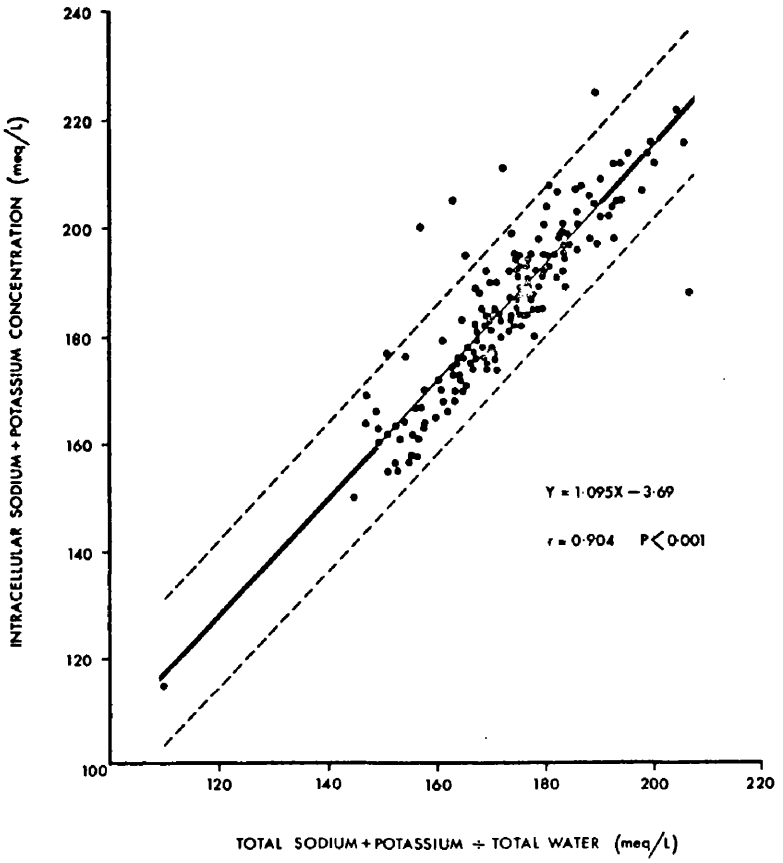

Figure 34: Correlation between total cation concentration and the sum of the intracellular sodium and potassium concentrations in the whole series.

close, both in normal subjects alone (Figure 33) and in the total series (Figure 34). The sum of the intracellular sodium and potassium concentrations is the major fraction of the total intracellular cation concentration, only magnesium contributing any further appreciable amount (about 17 - 20 meq. per litre under normal conditions[8]).

This therefore is good evidence in favour of osmotic control of cell water and electrolyte concentrations over a wide variety of pathological conditions, and also of the belief that there are no sustained osmotic gradients across the cell membrane[2-4].

DISCUSSION

The strong relationships found in this series between total water and the sum of the total sodium and potassium contents, between serum sodium concentration (and serum sodium plus potassium concentrations) and the total cation concentration, and between intracellular potassium concentration (and intracellular sodium plus potassium concentrations) and the total cation concentration, all favour the concept of osmotic homogeneity of the cells with the extracellular water. The relationships between the intracellular cation concentrations and the total cation concentration have never been reported before under abnormal conditions, although, Moore, et. al.[7] showed a similar correlation to that found in this

series between intracellular potassium concentration and total cation concentration in normal subjects. This therefore shows that the intracellular electrolyte concentrations in a wide range of clinical conditions are dependent on osmotic equilibrium between cells and extracellular fluid, in an exactly similar manner to the control of the serum electrolyte concentrations. Intracellular cation concentrations may therefore be predicted with considerable accuracy on the basis of total water, sodium and potassium contents, whether measured by muscle biopsy analysis or by isotope-dilution studies.

The regression equations found between various biopsy-specimen results as reported in this chapter are very similar to regression equations reported for the same parameters measured by isotope-dilution by Moore, et. al.[7]. This therefore, tends to confirm the beliefs stated in Chapter II of this thesis that the water and electrolyte values obtained by muscle biopsy analysis are an accurate reflection of total body water and electrolyte status.

Intracellular electrolyte concentrations are estimated in this study after partition of the total water content into extracellular and intracellular phases, which is carried out on the assumption that the biopsy-specimen chloride content is passively distributed between cells and extracellular fluid on the basis of a relatively constant cell membrane potential. The fact that the

intracellular electrolyte concentrations calculated in this way bear such a close correlation to the total cation concentration, a function of the total water, sodium and potassium contents of the biopsy-specimen, suggests that this method of partition of the water and electrolytes gives a good indication of the actual intracellular water and electrolyte concentrations of the patients at the time of biopsy, both under normal and abnormal conditions.

Finally, the close correlation between intracellular sodium plus potassium concentrations and the total cation concentration make it extremely unlikely that significant osmotic inactivation of cell cation occurs in the clinical conditions studied in this series. Elkinton, et. al.[9] studied the hypothesis of body fluid iso-osmolarity, and concluded that a significant and variable fraction of cell cation was osmotically inactive. Osmotic inactivation of cell cation has been postulated by other workers to explain discrepancies found between body water and cation content, especially in congestive cardiac failure, although this has not been found in other studies (see Chapter IX). The results of this present investigation are in agreement with the conclusions of Edelman, et. al.[4] that body water is passively distributed in proportion to osmotic activity, and that all or almost all of the body potassium is osmotically active.

SUMMARY

One hundred and sixty-seven biopsy-specimen results from 42 normal subjects and 117 ill patients have been analysed to assess the relationships between body water and electrolyte contents and concentrations.

Serum-electrolyte concentrations correlate only poorly with the appropriate total biopsy-specimen content or intracellular concentration.

Good correlations are found between the following sets of measurements:-

(i) total water to total sodium plus potassium contents.

(ii) extracellular water to total sodium content.

(iii) intracellular water to total potassium content.

(iv) serum sodium concentration to total cation concentration (the sum of the total sodium plus potassium contents divided by the total water).

(v) serum sodium plus potassium concentrations to total cation concentration.

(vi) intracellular potassium concentration to total cation concentration.

(vii) intracellular sodium plus potassium concentrations to total cation concentration.

The conclusions reached from these results are:-

(i) body water is passively distributed between cells and extracellular space according to osmotic activity, and that no sustained osmotic gradient exists across the cell membrane.

(ii) there is no significant osmotic inactivation of intracellular cation in the conditions studied in this series.

(iii) the technique of analysis and partition of the biopsy-specimen water and electrolytes appears to be accurate in a wide range of pathological conditions.

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MUSCLE BIOPSY ANALYSIS OF THE BODY WATER AND ELECTROLYTE CONTENT IN HEALTH AND DISEASE

SUBMITTED FOR THE DEGREE OF DOCTOR OF MEDICINE

by JOHN ALISTAIR GRAHAM, June, 1970

The work presented in this thesis describes the measurement of body water and electrolyte contents by analysis of muscle biopsy-specimens.

Biopsy-specimens were taken from skeletal muscle (usually vastus lateralis) under either local or general anaesthesia. Each biopsy-specimen is divided into three portions, which are weighed, dried and then weighed again. The total water content is thus obtained, and the total biopsy-specimen sodium, potassium and chloride content are then measured in an acid extract of the tissue. The total water and electrolyte contents are partitioned into extra- and intracellular phases using the chloride content of the biopsy-specimen (corrected for the intracellular content) as an indicator of the extracellular water. The first two chapters of the thesis give the historical background, the details of the method, and the advantages and possible disadvantages of this technique, The findings of the further chapters may be summarised thus:-

Chapter III Muscle water and electrolytes in normal adult subjects

The potassium content and intracellular potassium concentration of biopsy-specimens taken from vastus lateralis is slightly but significantly higher than that of biopsy-specimens taken from rectus

abdominis.

General anaesthesia does not affect muscle water and electrolyte contents.

There is a slight reduction in intracellular water and potassium content with increasing age.

Evidence is presented of the osmotic homogeneity of the body under normal conditions.

Chapter IV Muscle water and electrolytes in normal infants and children

Extracellular water, sodium and chloride contents are high in the newborn, falling almost to adult levels around 2 years of age, with probably a further slight fall till adolescence.

Intracellular water and potassium content are similarly high in the newborn, but fall to adult levels by six months of age.

Intracellular electrolyte concentrations are similar to adult values at all ages.

Chapter V Water and electrolyte imbalance in pyloric stenosis

Patients with chronic and subacute pyloric stenosis tend to show an expansion of extracellular water, sodium and chloride contents, with a loss of intracellular water and potassium; the typical findings of starvation.

In acute pyloric stenosis sodium and chloride contents are markedly reduced but the potassium content is normal. The osmotic gradient so produced across the cell membrane causes movement of extracellular water into the cell, thus accentuating the extracellular dehydration. Potassium loss is minimal in these patients, but potassium should be given during resuscitation to replace urinary losses.

Chapter VI Muscle water and electrolytes after ureterocolic anastomosis

All patients showed expansion of extracellular water, sodium and chloride contents, the usual finding in chronic renal failure.

Acutely ill patients had low biopsy-specimen potassium contents and had marked intracellular dehydration. Muscle potassium content was variable in well controlled patients.

Acutely ill patients require large amounts of potassium in addition to other resuscitative measures, but the value of long-term potassium therapy is doubtful.

Chapter VII The metabolic imbalance of chronic renal failure

Extracellular water, sodium and chloride contents are raised in chronic renal failure , the charges being more marked as the severity of renal impairment increases.

Intracellular water and electrolyte concentrations were

remarkably normal in these patients, suggesting that cellular function is very little upset.

Extracellular water content correlates well with the diastolic blood-pressure of the patients especially in more severe degrees of renal failure. This supports the belief that hypertension in patients with chronic renal failure is salt and water dependent.

Chapter VIII Body water and electrolyte composition in acute renal failure

Patients with acute untreated oliguric renal failure show two types of imbalance. The first group shows loss of extracellular sodium and chloride, with normal intracellular water and electrolytes. These findings are thought to be due to urinary salt loss during the development of oliguria and to inadequate replacement of extrarenal losses.

The second group shows generalised overhydration with an excess of sodium and chloride and a reduction in intracellular potassium concentration.

Hyponatraemia in such patients may therefore be due to either sodium deficit or water overloading, and separation of the two groups on clinical grounds is often difficult.

One patient studied during the diuretic recovery phase showed a marked loss of sodium and chloride, stressing the need to replace

urinary electrolyte losses at this time.

Chapter IX Muscle biopsy analysis in congestive cardiac failure

Severely ill oedematous patients have a gross increase in extracellular water, sodium and chloride contents. These measurements tend to be still greater than normal in non-oedematous patients.

There is a general tendency to a loss of potassium in these patient. In severely ill patients the intracellular potassium concentration was low, but it was normal in non-oedematous patients.

There was no evidence of osmotic inactivation of intracellular cation in the patients studied.

Chapter X The syndrome of inappropriate secretion of antidiuretic hormone

In untreated patients with this condition, extracellular water, sodium and chloride contents are greater than normal. Total potassium content and intracellular potassium concentrations are reduced.

After treatment by water deprivation, biopsy-specimen electrolyte contents remained as before. Extracellular water was reduced, although the values were still greater than normal. Intracellular water became significantly lower than normal, and as a result intracellular potassium coneentrations returned to normal.

The symptoms caused by this syndrome appear to be due to water

intoxication, i.e. by lowering of the intracellular potassium concentration.

Chapter XI Water and electrolyte metabolism in disorders of adrenal cortical function

1. Hyperaldosteronism: there is an expansion of extracellular water, sodium and chloride contents, and a loss of intracellular water and potassium content.

Intracellular potassium concentrations tend to be low and there is a consistent increase in intracellular sodium concentration. This has not been found in any other group of patients studied, and may be due to a direct effect of aldosterone on the cell membrane.

2. Cushing's Syndrome: two patients were studied. Both showed an increase in extracellular water, sodium and chloride contents, with a reduction in intracellular water and potassium content.

Intracellular electrolyte concentrations were quite normal in both patients.

3. Adrenocorticotrophin-secreting bronchogenic carcinoma: initial biopsy in a patient with condition revealed an expansion of extracellular water and electrolytes. Intracellular potassium concentration was low and intracellular sodium concentration was high.

After treatment with metyrapone extracellular water and electrolytes returned towards normal. Biopsy-specimen potassium content was markedly

increased. Metyrapone appears to have blocked production of mineralocorticoid hormones to the extent that excessive renal retention of potassium has occurred.

Chapter XII The relationships between extracellular and intracellular water and electrolytes in health and disease

Analysis of biopsy-specimen results from 42 normal patients and 117 ill patients (167 biopsy-specimens in all) shows that body water content correlates well with the electrolyte content.

Both extracellular and intracellular electrolyte concentrations are a function of the total body water and electrolyte contents.

The conclusions reached are:

(i) body water is passively distributed between cells and extracellular space according to osmotic activity, and that no sustained osmotic gradients exist across the cell membrane.

(ii) there is no significant osmotic inactivation of intracellular cation in the conditions studied in this work.

(iii) the close similarity between regression equations for the biopsy-specimen results and those obtained by other workers using isotope-dilution techniques is further evidence that muscle biopsy-specimen water and electrolytes give an accurate reflection of the body water and electrolyte status.